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Contents

Cancer Treatment Reports
Volume 67, Number 11
November 1983

Clinical and Preclinical Studies

967 Current Status of Amsacrine (AMSA) Combination Chemotherapy Programs in Acute Leukemia. **Z. A. Arlin.** New York Medical College, Valhalla

971 Human Neurophysins in Carcinoma of the Lung: Relation to Histology, Disease Stage, Response Rate, Survival, and Syndrome of Inappropriate Antidiuretic Hormone Secretion. **L. H. Maurer, J. F. O'Donnell, S. Kennedy, C. S. Faulkner, K. Rist, and W. G. North.** Dartmouth-Hitchcock Medical Center, Hanover, New Hampshire

977 Amsacrine in Refractory Adult Acute Leukemia: A Pilot Study of the Southeastern Cancer Study Group. **E. F. Winton, E. B. Hearn, W. R. Vogler, L. Johnson, T. Logan, and M. Raney.** Southeastern Cancer Study Group

981 Phase I Clinical Trial of 13-*cis*-Retinoic Acid in Myelodysplastic Syndromes. **E. J. Gold, R. H. Mertelsmann, L. M. Itri, T. Gee, Z. Arlin, S. Kempin, B. Clarkson, and M. A. S. Moore.** Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, New York

987 Phase I Investigation of Ametantrone. **D. M. Loesch, D. D. Von Hoff, J. Kuhn, C. A. Coltman, F. Tio, T. K. Chaudhuri, J. F. Bender, and A. J. Grillo-Lopez.** Wright-Patterson Air Force Base, Dayton, Ohio

993 Phase II Study of Aziridinylbenzoquinone in Refractory Lymphoma. **D. C. Case, Jr, and D. M. Hayes.** Maine Medical Center, Portland

997 Phase II Study of JM8, a New Platinum Analog, in Advanced Ovarian Carcinoma. **B. D. Evans, K. S. Raju, A. H. Calvert, S. J. Harland, and E. Wiltshaw.** The Royal Marsden Hospital, London, England

1001 Randomized Phase II Studies in Advanced Colorectal Carcinoma: A North Central Cancer Treatment Group Study. **H. Windschitl, M. Scott, A. Schutt, G. McCormack, L. Everson, S. Cullinan, J. Gerstner, J. Krook, J. Laurie, R. Shreck, R. Marschke, and D. Pfeifle.** North Central Cancer Treatment Group

1009 Phase II Trial of Lymphoblastoid Interferon in Metastatic Colon Carcinoma. **T. Chaplinski, J. Laszlo, J. Moore, and P. Silverman.** Duke University Medical Center, Durham, North Carolina

1013 Lack of Dexamethasone Effect on the Antitumor Activity of Cisplatin. **M. S. Aapro, D. S. Alberts, and R. Serokman.** University of Arizona, Tucson

1019 Effect of Temperature on the Cytotoxicity of Vindesine, Amsacrine, and Mitoxantrone. **T. S. Herman.** Veterans Administration Hospital, Little Rock, Arkansas

BRIEF REPORTS

1023 Etoposide-Induced Hepatic Injury: A Potential Complication of High-Dose Therapy. **D. H. Johnson, F. A. Greco, and S. N. Wolff.** Vanderbilt University School of Medicine, Nashville, Tennessee

1025 Fatal Acute Respiratory Failure Following Intrathecal Methotrexate Administration. **J. E. Hamous, M. M. Guffy, and C. A. Aschenbrener.** University of Iowa, Iowa City

1027 Phase I Trial of PCNU. **L. A. Kalman, R. J. Gralla, E. S. Casper, M. G. Kris, C. Gordon, and T. M. Woodcock.** Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, New York

1031 Iv Administration of a Water-Insoluble Antimitotic Compound Entrapped in Liposomes. Preliminary Report on Infusion of Large Volumes of Liposomes to Man. **A. Coune, J. P. Sculier, J. Frühling, P. Stryckmans, C. Brassinne, G. Ghanem, C. Laduron, G. Atassi, J. M. Ruysschaert, and J. Hildebrand.** Institut Jules Bordet, Brussels, Belgium

1035 Cerebrospinal Fluid-Platinum Kinetics of Cisplatin in Man. **J. P. Armand, J. P. Macquet, and A. F. LeRoy.** Centre Claudius Regaud, Toulouse, France

CLINICAL TRIALS SUMMARIES

1039 Phase II Study of Mitoxantrone in Patients With Metastatic Breast Carcinoma: A Southeastern Cancer Study Group Project. **R. Smalley and R. Gams.** Southeastern Cancer Study Group

1041 Phase II Evaluation of Vindesine in Non-Small Cell Bronchogenic Carcinoma. **A. W. Hutcheon, J. B. D. Palmer, M. A. Pratt, and R. A. Clark.** Aberdeen Royal Infirmary, Scotland

1043 Phase II Study of Amsacrine in Metastatic Renal Cell Carcinoma: A Cancer and Leukemia Group B Study. **P. C. Amrein, M. Coleman, F. Richards, II, R. F. Poulin, I. Berkowitz, B. J. Kennedy, M. Green, R. Herschkopf, and S. Rafla.** Cancer and Leukemia Group B

1045 Maytansine in Refractory Childhood Acute Lymphocytic Leukemia: A Pediatric Oncology Group Study. **H. Sabio, L. Frankel, C. Sexauer, J. Falletta, and T. H. Kim.** Pediatric Oncology Group

1047 Phase II Clinical Trial of L-Alanosine in Advanced Upper Aerodigestive Cancer. **E. T. Creagan, A. J. Schutt, J. N. Ingle, and J. R. O'Fallon.** Mayo Clinic and Mayo Foundation, Rochester, Minnesota

LETTERS

1049 Treatment of Side Effects in Patients Receiving Iv Metoclopramide. **S. B. Strum and J. E. McDermed.** University of Southern California, Los Angeles

1050 Stevens-Johnson Syndrome Associated With Etoposide Therapy. **C. H. Jameson and D. L. Solanki.** University of Oklahoma Health Sciences Center, and Veterans Administration Medical Center, Oklahoma City

1051 High-Dose Tamoxifen in Metastatic Melanoma. **R. J. Papac and J. M. Kirkwood.** Veterans Administration Medical Center, West Haven, Connecticut

1053 ANNOUNCEMENTS

Current Status of Amsacrine (AMSA) Combination Chemotherapy Programs in Acute Leukemia[1]

Zalmen A. Arlin[2,*]

The identification of new active agents for the treatment of acute leukemia and their incorporation into combination chemotherapy programs is an important element in the development of improved total therapy programs for acute leukemia. Amsacrine (AMSA) is an acridine derivative with proven utility in patients with acute nonlymphoblastic leukemia (ANLL) and acute lymphoblastic leukemia (ALL) in relapse (1,2). In ANLL, at doses ranging from 450 to 1000/mg/m^2/course, remission rates ranging from 17% to 28% have been seen (1-6). In ALL, at doses ranging from 225 to 1200 mg/m^2/course, remission rates ranging from 9% to 42% have been seen (1,2,4,5,7-9).

In previously treated patients who achieved remission with AMSA, the remissions were generally brief and, occasionally, they lasted as long as 1 year (1,2), but the median remission duration was approximately 4 months. For this reason, efforts have been taken to explore the activity and toxicity of AMSA in combination with other active antileukemic agents. The purpose of this report is to update the current status of these programs.

Toxicity of AMSA

The toxicity of AMSA has been well-defined. Nausea and vomiting are common. The occurrence of stomatitis is predictable in both degree and duration and relates specifically to the dose employed. At doses of < 600 mg/m^2/course, stomatitis is mild and occurs in only approximately one-third of patients. At doses of 1000 mg/m^2/course, stomatitis is virtually universal and may lead to severe dysphagia, inability to swallow, and oral hemorrhage. Hyperbilirubinemia occurs in one-fourth to one-third of patients and, characteristically, is not associated with elevation of liver enzymes. In most instances, there is elevation of both direct and indirect fractions, and the hyperbilirubinemia is usually reversible. When AMSA is administered via a peripheral vein, it may cause severe phlebitis; however, administering the drug through a central indwelling catheter eliminates this complication. Cardiac arrhythmias or seizures have rarely been associated with administration of the drug, and their occurrence is closely associated with electrolyte abnormalities or other predisposing conditions, such as CNS leukemia and sepsis. Correction of hypokalemia eliminates the risk of cardiac arrhythmia. The relationship of hypokalemia to seizure activity remains more obscure.

AMSA in Previously Treated ANLL

Table 1 outlines the combination chemotherapy programs incorporating AMSA for therapy of ANLL (10-16).[3,4] The studies were done primarily in previously treated adults. It is important to note that AMSA was usually given over 3-5 days, at doses ranging from 210 mg/m^2/course to as high as 1000 mg/m^2/course. AMSA has been combined with either cytarabine, cytarabine and thioguanine, cytarabine/vincristine/prednisone, cytarabine/etoposide and thioguanine, or with azacitidine or cyclocytidine. The toxicity of the combinations has been quite predictable. When the highest doses of AMSA were combined with cytarabine, unacceptable toxic effects of severe enteritis and stomatitis developed (16). When lower-dose AMSA was combined with high-dose cytarabine (15), the most prominent side effects related more to high-dose cytarabine than to AMSA, and included nausea and vomiting (90% of patients), ocular discomfort (60%), mucositis (73%), hepatic dysfunction (25%), cutaneous erythremia (75%), and cerebellar dysfunction (23%). The major toxic effect caused by azacitidine was severe nausea (100% of patients), and the major toxic effects caused by AMSA were hepatic dysfunction (32%) and stomatitis (57%). The most widely reported experience of AMSA in combination is with cytarabine either alone or with the addition of a third or fourth drug. These programs (10,11,13,15)[3] were generally well-tolerated, and hepatic dysfunction was not worse than with AMSA alone.

[1]Received Jan 13, 1983; revised May 23, 1983; accepted June 7, 1983.
[2]Division of Neoplastic Diseases, New York Medical College, Valhalla.
[3]J. Malpas, personal communication.
[4]E. Winton, personal communication.
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TABLE 1.—AMSA combination chemotherapy programs for ANLL

Author	AMSA (mg/m^2/course)	Other drugs (mg/m^2)	No. of patients with complete remission/ total patients (%)
Arlin et al (ref 10)	600–740	Cytarabine, 200/day × 5 Thioguanine, 100 every 12 hrs × 10	8/25 (32)
Malpas* (footnote 3)	500	Cytarabine, 100/day × 7	6/15 (40)
McCredie et al* (ref 11)	210	Cytarabine, 70/day × 7 Vincristine 2/day × 1 Prednisone, 100/day × 5	26/56 (46)
Kahn et al (ref 12)	750	Azacitidine, 150/day × 5	7/14 (50)
Hurd et al (ref 13)	675	Etoposide, 200/day × 3 Cytarabine, 200/day × 3 Thioguanine, 100–200/day × 3	9/17 (53)
Miller et al† (ref 14)	375	Cyclocytidine, 600/day × 7	
Hines et al (ref 15)	225–300	Cytarabine, 3000 every 12 hrs × 12	28/40 (70)
Winton (footnote 4)	300–600	Azacitidine, 112–300/day × 4	4/45 (9)
Weil‡ (ref 16)	120–1000	Cytarabine, 200/day × 6	3/12 (25)

*Previously untreated patients.
†Children.
‡Children and adults.

AMSA in Previously Treated ALL

Table 2 shows the result of AMSA combination programs in previously treated patients with ALL. The experience is much less extensive (10,14,16) than with ANLL; however, important activity has also been noted.

In many of the AMSA combination programs, the complete remission rate is higher than the rate reported for AMSA alone. Even if this were not the case, however, it would still be appropriate to consider these combination programs for front-line therapy. It should be noted that virtually all patients receiving these combinations had previously received the other elements of the combination and, in many instances, were probably resistant to them. The purpose of these trials is to show that the combinations are well-tolerated and that the complete remission rate is no worse than that seen with AMSA alone.

AMSA in Previously Untreated Leukemia

To fully evaluate the place of AMSA-based regimens in the treatment of leukemia, it is necessary to compare these regimens in previously untreated patients with standards currently in use. For ANLL, this has already been done at Memorial Sloan-Kettering Cancer Center, where a comparable remission induction rate has been seen using AMSA plus cytarabine/thioguanine versus daunorubicin plus cytarabine/thioguanine (17). The Mid-Atlantic Oncology program has recently begun a similar trial. At M. D. Anderson (11), a randomized trial has not been done, but using a highly predictive model for remission induction, it was shown that AMSA, vincristine, prednisone, and cytarabine induced a higher (than anticipated) remission rate in patients with ANLL who have a $< 40\%$ likelihood of achieving remission with a similar doxorubicin-containing regimen.

TABLE 2.—AMSA combination chemotherapy programs for ALL

Author	AMSA (mg/m^2/course)	Other drugs (mg/m^2)	No. of patients with complete remission/ total patients (%)
Arlin et al (ref 10)	600–740	Cytarabine, 200/day × 5	3/13 (23)
Miller et al† (ref 14)	375	Cyclocytidine, 600/day × 7	3/5* (60)
Weil (ref 16)	120–1000	Cytarabine, 200/day × 5	17/37† (46)

*Children and adults.
†Children.

Future Directions

The results of the trials reported here point to several other clinical questions worthy of exploration. High-dose cytarabine is active as a single agent. Whether AMSA improves the high remission rate that is possible with high-dose cytarabine (18) remains unclear. A randomized trial comparing the remission rate and toxicity with AMSA plus high-dose cytarabine versus high-dose cytarabine alone appears warranted. A high remission rate has been seen by at least one center using AMSA in combination with azacitidine (12), but not in another trial.[4] Further evaluation of this combination as primary therapy in ANLL may also be indicated.

The most important contribution of these programs may be the development of non-cross-resistant combinations. One can conceive of remission induction with an anthracycline in combination with cytarabine, followed by intensification therapy with various combinations that could include AMSA, high-dose cytarabine, azacitidine, and etoposide. It is possible that the beneficial effect of non-cross-resistant combinations established so well in Hodgkin's disease (19,20) may be applicable to the situation with ANLL and may translate into an improved cure rate.

In ALL, the situation is more difficult, in view of the high remission rate possible with standard agents. Therefore, it will be necessary to limit these combination chemotherapy programs to the period after remission induction. Prognostic features in patients with ALL are now undergoing clarification. Initially, it may be appropriate to explore the role of AMSA combinations in patients with high-risk ALL (21).

It is important that we rapidly take advantage of the opportunity afforded by the introduction of AMSA, and by the emergence of the effective combination chemotherapy programs which incorporate AMSA. Further studies should define how these combinations will fit into "total therapy" programs in the acute leukemias.

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Human Neurophysins in Carcinoma of the Lung: Relation to Histology, Disease Stage, Response Rate, Survival, and Syndrome of Inappropriate Antidiuretic Hormone Secretion[1,2]

L. Herbert Maurer,* Joseph F. O'Donnell, Steven Kennedy, Charles S. Faulkner, Kevin Rist, and William G. North[3]

At diagnosis, 65% of 103 patients with small cell carcinoma of the lung were found to have elevated plasma concentrations of vasopressin-associated human neurophysin (VP-HNP), oxytocin-associated human neurophysin (OT-HNP), or both, which were thought to be related to tumor secretion of these proteins. The remainder of patients were designated as nonsecretors (24%) or possible secretors (11%), depending upon plasma concentration of the neurophysins prior to therapy. There was a significantly higher percentage of secretors among patients with extensive disease (82%) than among those with limited disease (40%) ($P = 0.001$). However, within each stage group, there was no correlation between secretory status and response to therapy, survival, or histologic subtype. In addition, patients who initially were nonsecretors or possible secretors maintained this status throughout the course of disease remission and subsequent relapse. These findings suggest the possibility of biochemical differences between tumors which present as limited disease and those which present as extensive disease. The syndrome of inappropriate antidiuretic hormone secretion (SIADH) was infrequent in limited disease but was present in 33% of patients with extensive disease. SIADH was not seen without VP-HNP elevation; however, with extensive disease, 49% of patients with elevated VP-HNP had SIADH. In contrast, elevated plasma concentrations of the neurophysins were seen in only 19.6% of 56 patients with non-small cell carcinoma of the lung. The levels were in general lower than those in patients with small cell carcinoma and were seen at approximately equal frequencies in each major cellular subtype. [Cancer Treat Rep 67:971–976, 1983]

Major advances in the therapy of small cell carcinoma of the lung (SCCL) have resulted from combination chemotherapy and radiation therapy. Despite these advances it is often difficult to define a response accurately (especially when a patient has achieved complete remission), to detect residual disease, or to identify impending relapse prior to recognition of gross disease. The neurophysins, which are part of the precursor structure of vasopressin and oxytocin, also act as carrier molecules for these hormones (1). Each hormone has its own specific neurophysin and each is synthesized normally in a different cell in the magnocellular system of the hypothalamus (2). Previous studies at this institution have suggested that measurement of plasma vasopressin-associated and oxytocin-associated human neurophysins (VP-HNP and OT-HNP) may be useful markers in the management of this disease (3). The purpose of this report is to examine further the use of plasma elevations of neurophysins as peptide markers in SCCL, their relationship to the syndrome of inappropriate antidiuretic hormone secretion (SIADH), and their possible use as prognostic indicators. Further, we explored the possibility that measurement of these peptides may be of value in determining biologic differences in cell types unrelated to the current histologic classification.

To investigate the specificity of neurophysins as

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"markers" for small cell anaplastic carcinoma, we have studied their levels in patients with other types of lung cancer.

METHODS AND MATERIALS

Small Cell Lung Cancer

Plasma VP-HNP and OT-HNP were determined at the Dartmouth-Hitchcock Medical Center, by radioimmunoassay techniques during the initial staging of 103 patients with SCCL and at periodic intervals prior to each cycle of chemotherapy or radiation therapy (4). Each patient had a histologic or cytologic diagnosis of SCCL and was evaluated for sites of metastatic disease using physical examination; chest x-ray; bone marrow aspirates or biopsies; radionuclide scans of liver, bone, and brain; or computerized axial tomography of the brain. Staging was assessed in accordance with Cancer and Leukemia Group B (CALGB) criteria, which define patients with limited disease as those with tumor confined to the lung, mediastinum, and ipsilateral or contralateral supraclavicular lymph nodes; all other patients had extensive disease (5).

All patients were treated with combination chemotherapy or chemotherapy plus radiation therapy to the primary tumor. Many patients also received prophylactic whole-brain radiation therapy. A complete remission (CR) was defined as disappearance of all measurable tumor and all laboratory or radiologic evidence of disease. A partial response (PR) was defined as a 50% reduction in the sum of the products of two diameters of all measurable tumors. Nonresponders had to have had a $< 50\%$ decrease in tumor measurements and no objective progression.

Non-Small Cell Lung Cancer

Fifty-six patients with non-small cell lung carcinoma (NSCLC) were studied, 39 at the time of diagnosis and 17 at the time of disease progression, at least 2 months after initial therapy. No patients were undergoing antineoplastic therapy at the time the samples were obtained.

Patient Classification

Patients were divided into three categories of presumed tumor secretory status: (*a*) nonsecretors had plasma neurophysins that were less than two times the mean for the control population, (*b*) possible secretors had plasma neurophysins between two and three times above normal, and (*c*) secretors had plasma neurophysins that were three or more times the mean of the normal population. Normal plasma values used for VP-HNP and OT-HNP were 73 ± 30 pg/ml and 283 ± 30 pg/ml, respectively (3). Therefore, secretors are defined as having > 220 pg/ml of VP-HNP and > 850 pg/ml of OT-HNP. A blinded systematic review of all biopsy and autopsy material was undertaken in an attempt to correlate subtypes of SCCL and secretory status as defined above. This review and the review of the cases of NSCLC were performed by the CALGB central reference pathologist (C. S. Faulkner) in accordance with the World Health Organization classification (6). Comparisons were made between initial secretory status (secretor, nonsecretor, possible secretor) and stage of disease, response to therapy, survival, cell subtype, and incidence of SIADH. SIADH is defined as plasma hypo-osmolality; urinary osmolality greater than plasma; continued urinary excretion of sodium; absence of volume depletion; and normal renal, adrenal, and thyroid functions (7). A longitudinal study was made of 42 patients with SCCL for whom four or more samples were studied throughout the course of disease. Twenty-two patients were secretors, five were possible secretors, and 15 were nonsecretors.

RESULTS

Small Cell Lung Cancer

Of the 103 patients studied, 67 (65%) were secretors, 25 (24%) nonsecretors, and 11 (11%) possible secretors. For human neurophysin-secreting tumors, 51% were producers of VP-HNP alone, 19% were producers of OT-HNP alone, and 30% produced an elevation of both neurophysins. With respect to staging, 82% of patients with extensive disease were secretors, whereas only 40% of those with limited disease had secreting tumors (table 1).

The frequency of SIADH for the study population was 22%, but it was seen much more frequently in extensive disease than in limited disease (33% vs 7%; $P = 0.005$). As noted in table 2, nearly one-half of patients with extensive disease who had elevated VP-HNP had SIADH, as compared to 23% with limited disease. The VP-HNP concentration was significantly greater in patients with SIADH ($P = 0.01$), when compared with VP-HNP secretors who did not have SIADH (fig 1). The ultimate cause of SIADH is an inappropriate level of antidiuretic hormone (ADH), and a determination of ADH levels in these same samples is being undertaken.

There was a good correlation between decrease in neurophysin levels and response to therapy in secretors with limited and extensive disease. Sixteen of the 22 patients who were defined as secretors and who had four serial samples evaluated had either a CR or a PR. Fifteen of these 16 patients had a corresponding decrease in plasma neurophysin values with response to therapy. The remaining patient achieved a CR, but, despite a large decrease in VP-HNP levels, a small increase in OT-HNP persisted until relapse. Of the four patients who had no clinically detectable response, three had small decreases in plasma neurophysin levels followed later by an increase

TABLE 1.—Frequency of human neurophysin production related to stage of disease in SCCL

Secretory status	No. of patients/total tested (%)		
	Limited disease	Extensive disease	Total
Secretors	17/42 (40)	50/61 (82)	67/103 (65)
VP-HNP	10/17 (59)	24/50 (48)	34/67 (51)
OT-HNP	4/17 (24)	9/50 (18)	13/67 (19)
VP-HNP + OT-HNP	3/17 (17)	17/50 (34)	20/67 (30)
Nonsecretors	16/42 (39)	9/61 (15)	25/103 (24)
Possible secretors	9/42 (21)	2/61 (3)	11/103 (11)

at the time of disease progression. Of the two patients with progressive disease, one had a temporary decrease in neurophysin levels prior to an increase.

Of the 20 patients who had nonsecreting tumors or possibly secreting tumors, 16 retained the same secretory status throughout the course of their disease. Three patients had increases of VP-HNP coinciding with the onset of other problems which may be associated with increases in this protein, ie, massive ascites with disease relapse, unexplained cerebral deterioration without evidence for recurrent SCCL, and in one patient a sample was drawn just prior to death. The fourth patient has remained in CR for 3 years with slightly elevated levels of VP-HNP.

Table 3 shows the relationships between secretory status of the tumor, response to therapy, and median survival of patients. There was no significant difference in the response of tumors to therapy or in median survival when secretors and nonsecretors were compared. Of the four 2-year survivors, two were nonsecretors, one was a possible secretor, and one was a persistent secretor. There was also no clear association of neurophysin-secreting tumors with any particular histologic cell subtype.

Non-Small Cell Lung Cancer

In lung cancer other than SCCL, elevations of neurophysin were seen in 11 of 56 patients (19.5%) (table 4). While the elevated values of VP-HNP ranged from 220 to 45,113 pg/ml (median, 528) in SCCL, they ranged from 220 to 1029 pg/ml (median, 350) in NSCLC. Seventy percent of the values for the patients with SCCL were above the median value of 350 for NSCLC. For OT-HNP, elevated values ranged from 850 to 5239 pg/ml (median, 1122 pg/ml) in SCCL and from 933 to 3339 pg/ml (median, 1261 pg/ml) in NSCLC. The distribution of values in NSCLC is contrasted to that of SCCL in figure 2. Hyponatremia was seen in three of the 56 patients with NSCLC. Only one of these three had elevated VP-HNP. In the other two patients, the cause of the hyponatremia was not clearly SIADH; however, assays of ADH are in progress.

DISCUSSION

The use of peptide hormone markers as indicators of human tumor response has been the subject of considerable interest and much debate in the past few years. With the exceptions of calcitonin in medullary carcinoma of the thyroid and human chorionic gonadotropin and alpha-fetoprotein in germ cell tumors and hepatomas, the assays for peptide hormones have had neither good sensitivity nor specificity.

Richardson et al (8) and Hansen (9) have extensively reviewed the paraneoplastic syndromes in SCCL and the potential use of peptide hormones as markers. The hormones most frequently detected in the peripheral blood are ACTH, ADH, and calcitonin. To date, no single marker peptide measured prior to treatment has proven to be of value in predicting stage of disease, response to therapy, or long-term survival. Because of the inconsistency of results, most investigators have questioned the reliability of the peptides as measures of tumor response. Data obtained from long-term cultures of many cell lines from SCCL indicate that tumor cells are capable of producing and releasing a much broader spectrum of hor-

TABLE 2.—VP-HNP with relation to SIADH

	Limited disease	Extensive disease	Total
No. of patients/total tested (%) with—			
SIADH	3/42 (7)	20/61 (33)	23/103 (22)
Elevated VP-HNP	13/42 (30)	41/61 (67)	54/103 (52)
Elevated VP-HNP who have SIADH	3/13 (23)	20/41 (49)	23/54 (43)

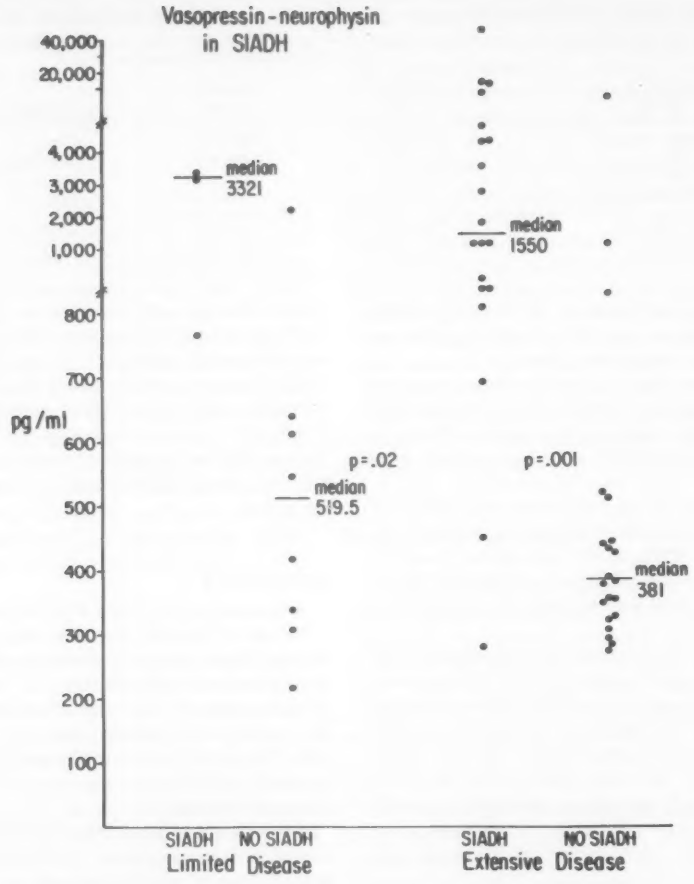

FIGURE 1.—VP-HNP plasma concentrations in patients with and without SIADH.

mones than previously suspected (10,11). In some cases, a single tumor has produced as many as 12 hormones in vitro (G. D. Sorenson, personal communication). Whether multiple hormone secretion occurs in vivo is unknown.

The 65% incidence of neurophysin production by SCCL in the present study is consistent with other studies which have measured serum ADH (9) or response to water loading (12). The incidence of neurophysin production in SCCL is more common with extensive than with limited disease. In an earlier study, Hansen et al (13) could find no correlation between secretion of hormones and stage of disease when they screened for 18 peptide hor-

TABLE 3.—Neurophysin secretory status: relation to response rate and survival

Secretory status	Limited disease*			Extensive disease*		
	CR	PR	Median survival (mos)	CR	PR	Median survival (mos)
Secretor	11/17 (65)	4/17 (24)	9.3	4/50 (8)	14/50 (28)	6.1
Nonsecretor	10/16 (69)	2/16 (13)	9.5	1/9 (11)	3/9 (33)	6.5
Possible secretor	2/9 (22)	7/9 (78)	10.0	1/2 (50)	0/2 (0)	5.8

* Values for CR and PR = No. of patients/total tested (%).

TABLE 4.—Neurophysins in NSCLC

	No. of patients				
	Total	Prior therapy*	Elevated neurophysins	VP-HNP (pg/ml)	OT-HNP (pg/ml)
Squamous cell carcinoma	29	23 none, 6 RT	4	252	
				243	
					933
					1020
Adenocarcinoma	17	10 none, 5 RT, 2 chemotherapy	5	1098	1261
				582	1776
				220	
				298	
				350	
Large cell carcinoma	10	6 none, 4 RT	2	365	
					3339

*RT = Radiation therapy.

mones or amine metabolites. However, Wallach et al (14) have recently shown a high incidence (84%) of plasma calcitonin production by SCCL in patients with extensive disease prior to therapy and no elevations in patients with limited disease, suggesting that the detection of this tumor marker is a function of tumor bulk. Moreover, using water loading tests, Comis et al (12) have found the frequency of abnormal tests to be greater in extensive disease (87%) than in limited disease (36%), with similar implications.

While our data show a similar relationship between markers and staging, we believe that factors other than tumor bulk may account for this distribution. Most patients with nonsecreting tumors prior to therapy main-

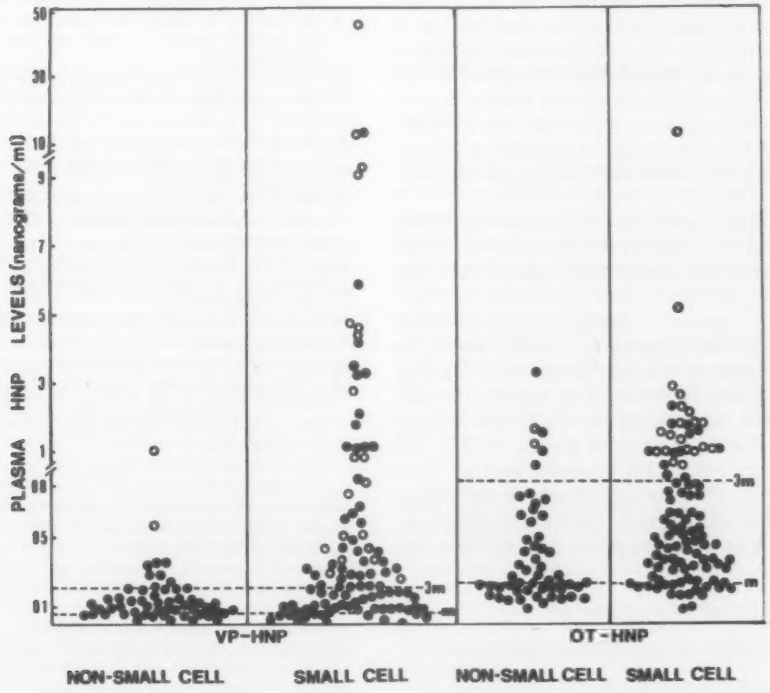

FIGURE 2.—Plasma VP-HNP and OT-HNP concentrations in patients with NSCLC and SCCL.

tained this status throughout the course of disease, despite disease progression. This indicates to us the possibility that limited SCCL and extensive SCCL are distinct biochemical subtypes. The calcitonin data presented by Wallach et al (14) support this argument, in that only one of their patients with limited disease changed from nonsecretory to secretory status. The category which we define as possible secretors behave more like nonsecretors, in that levels of markers remain unchanged despite the clinical course of the disease. It suggests that these slightly increased levels of neurophysins reflect events other than ectopic release of hormone.

This study demonstrates that, in contrast to SCCL, elevated levels of neurophysins in NSCLC are much less frequent (65% vs 19.6%). Furthermore, the increased levels seen are much lower than those seen in SCCL, and in two of 11 NSCLC patients with increased levels, pathologic elements consistent with SCCL were seen.

Although heterogeneity of tumor marker production has been seen at different metastatic sites for several enzymes or hormone markers of SCCL (15), we have found that this does not generally apply for the neurophysins. In limited studies, no differences in amounts of neurophysins between primary and metastatic tumor deposits have been found (3). Other than the different incidences of neurophysin production by stage of disease, there are no prognostic differences in response rate and survival between bearers of tumors that secrete and bearers of those that do not secrete. Also, we found no difference in secretory status among the histologic subtypes of SCCL. Unfortunately, this study has involved a number of overlapping therapeutic protocols, which may obscure these data. The CALGB is now evaluating this peptide marker prospectively in protocols for both limited and extensive disease.

Although VP-HNP itself is not responsible for SIADH, in every instance of SIADH in this study, VP-HNP was elevated. The levels were significantly higher for patients with SIADH.

Correlation of response to therapy and rise or fall of neurophysins continues to support our earlier data (3). In fact, in some instances, although there was no evidence of a clinical response, there were brief decreases in plasma neurophysins, suggesting that cell death had occurred. In the future, analysis of plasma half-life and secretory rate per cell for the neurophysins may allow the plotting of tumor volume and disease regression similar to the models developed by Salmon and Smith for myeloma (16). Further investigation with multiple hormone markers, perhaps the neurophysins, calcitonin, and ACTH (or the products of this complex protein), may lead to a clearer understanding of the biology of SCCL, the frequency of hormone secretion, and the potential of such hormones as markers in this common lung cancer.

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Amsacrine in Refractory Adult Acute Leukemia: A Pilot Study of the Southeastern Cancer Study Group[1,2,3]

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A phase II pilot study of amsacrine in refractory adult acute leukemia was conducted by the Southeastern Cancer Study Group from May 1979 to August 1980. Amsacrine, 90 mg/m², was given daily for 5–8 days to 45 patients with acute myeloblastic leukemia, 15 patients with acute lymphoblastic leukemia, and six patients with blastic transformation of chronic granulocytic leukemia. Of the 66 patients entered in the study, 59 (89%) were evaluable for response. Complete remissions were observed in eight of 41 evaluable patients with acute myeloblastic leukemia (20%) and in three of 12 with acute lymphoblastic leukemia (25%). Remissions were short-lived (median, 7.9 weeks; range, 2–27). Toxic effects included the expected myelosuppression (100%), as well as moderate to severe stomatitis (46%), hyperbilirubinemia (30%), and supraventricular tachycardia (l.5%). This cooperative group pilot study confirms previous reports from single institutions that amsacrine is a useful drug in the treatment of refractory adult acute leukemia and is worthy of further study. [Cancer Treat Rep 67:977–980, 1983]

In the initial clinical trials with amsacrine, myelosuppression was identified to be the dose-limiting toxic effect, and an antileukemic effect was noted (1–7). Based on these observations, the Southeastern Cancer Study Group (SECSG) began a phase II study in May 1979 using amsacrine as a single agent in adults with acute leukemias refractory to conventional therapy. An interim report of this trial was subsequently submitted (8). Over a 15-month period, 66 patients were entered in the study. The therapeutic results and toxic effects noted in this large group of patients treated with a uniform regimen confirm the activity of amsacrine in refractory myeloid (5–7) as well as lymphoid (5,7) acute leukemias and emphasize the frequent occurrence of hyperbilirubinemia, stomatitis, and the expected myelosuppression.

MATERIALS AND METHODS

Patients

Criteria for entry into the protocol included the diagnosis of acute myeloblastic leukemia (AML), acute lymphoblastic leukemia (ALL), or blastic transformation of chronic granulocytic leukemia (BT-CGL) resistant to established therapy. Also required were the absence of residual toxic effects of prior chemotherapy and a Karnofsky performance status > 40%.

Between May 1979 and August 1980, 45 patients with AML, 15 patients with ALL, and six patients with BT-CGL were entered in this phase II trial. Patients ranged in age from 15 to 69 years (mean, 37). There were 30 fe-

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[2]Supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated: University of Alabama, Birmingham (CA-03013 and CA-24456); University of Cincinnati, OH (CA-28138); Duke University, Durham, NC (CA-03177); Emory University, Atlanta, GA (CA-03227); Case Western Reserve University, Cleveland, OH (CA-15584); University of Kentucky, Lexington (CA-20255); University of Puerto Rico, San Juan (CA-12223); Rush Presbyterian St. Luke's Medical Center, Chicago, IL (CA-12640); St. Louis University, MO (CA-17214); University of Tennessee Memorial Research Center, Knoxville (CA-13237); Vanderbilt University, Nashville, TN (CA-23909); and Washington University, St. Louis, MO (CA-03376).

[3]The amsacrine used for these studies was provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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male and 36 male patients. Only two patients had not achieved a remission with conventional therapy prior to the trial with amsacrine. Twenty-seven of 29 patients in first relapse did not receive amsacrine as first-line therapy for reinduction; they had previously failed one to five courses of other chemotherapy.

Of the 45 patients with AML, 24 had failed re-induction with an anthracycline and 11 had relapsed within 3 months of receiving an anthracycline. Thus, 35 (78%) of the 45 AML patients were considered resistant to anthracyclines. All patients with BT-CGL and ALL had received vincristine-prednisone combinations as primary therapy, and eight of the 15 patients with ALL had previously received L-asparaginase.

Treatment Regimen

Patients received 90 mg/m^2 of amsacrine in 300 ml of 5% dextrose in water in a 3-hour iv infusion daily for 5 days. The protocol was revised subsequently to permit 3 additional days of amsacrine at the above dosage in patients who, on Day 5 of treatment, had marrow with > 50% cellularity containing > 50% leukemic cells. Nine patients received this extended course. A bone marrow aspirate and biopsy were required on Day 15 after therapy was started, and repeat courses of amsacrine were administered at that time if the marrow was > 50% cellular with > 30% abnormal cells. If a patient had hypocellular bone marrow at Day 15, the marrow examination was repeated every week; if leukemic cells were apparent, another course of amsacrine was given.

Patients who achieved a complete remission (CR) were given maintenance therapy with a single dose of amsacrine (150 mg/m^2) every 3-4 weeks, as tolerated.

Evaluation of Response and Toxicity

All patients who received a complete course of therapy and had one posttreatment marrow examination were considered evaluable for response. Patients were evaluated for CR and partial remission (PR) using standard criteria of the Acute Leukemia Group B for evaluation of acute leukemia. CR was defined as a normally cellular marrow of < 5% blasts with < 10% total blasts and promyelocytes (AML) or < 40% total blasts and lymphocytes (ALL), a granulocyte count > 2000/μl, and a platelet count > 100,000/μl. PR was defined as a normally cellular marrow with between 5.1% and 25% blasts, a granulocyte count > 1000/μl, and a platelet count > 50,000/μl.

Patients who had follow-up serum chemistries and blood cell counts were evaluable for toxicity. Possible renal and hepatic toxic effects were monitored by weekly blood chemistries, and the observed toxic effects were graded according to standard SECSG criteria.

RESULTS

Therapeutic

Fifty-nine of the 66 patients (89%) were considered evaluable for response. Of the evaluable patients, 19% (11 of 59) achieved a CR, and 10% (six of 59) a PR. As shown in table 1, CR were observed in both AML and ALL. In table 2, the clinical outcomes of all patients are categorized according to whether they displayed drug resistance (posttreatment marrow remained hypercellular with a predominance of leukemic cells), showed relative drug resistance (posttreatment marrow became hypocellular with a persistence of leukemic cells), or acquired aplastic marrow (posttreatment marrow was hypocellular with no recognizable leukemic cells). Of all evaluable patients, 58% displayed relative or absolute drug resistance. On the other hand, marrow aplasia was achieved in 25 (42%) of the evaluable patients. Approximately 25% of these patients were potential candidates to respond favorably but died while aplastic, while only two of the 25 patients had marrow that regenerated with a predominance of leukemic cells.

Eight of the nine patients who received the extended course of amsacrine were evaluable for response. Three patients had a transient PR, while the remaining five displayed relative or absolute drug resistance.

While CR were obtained after single courses of amsacrine with this schedule and dosage, more than one course was usually required (mean, 2.6 courses; range, 1-4). Remissions were short-lived (median, 7.9 weeks; range, 2-27), despite attempts to maintain remissions with maintenance therapy. One patient died with septicemia related to myelosuppression from maintenance chemotherapy.

The characteristics of patients with AML who achieved CR or PR were not different from those of the entire study group. Of interest were 11 of 12 patients with AML who achieved remission and who were anthracycline-resistant, seven having failed cytarabine and anthracycline treatment just prior to receiving amsacrine, and four having relapsed within 3 months of receiving an anthracycline. The remaining patient had received the dose limit of anthracycline. The number of relapses and the number of courses of other re-induction therapy tried before the amsacrine trial were identical to those for the whole study group.

TABLE 1.—Therapeutic response to amsacrine

Diagnosis	No. of patients		
	Entered	Evaluable	CR (%)
AML	45	41	8 (20)
ALL	15	12	3 (25)
BT-CGL	6	6	0 (0)
Total	66	59	11(19)

TABLE 2.—Clinical outcome with amsacrine

	No. of patients			
	AML	ALL	BT-CGL	Total (%)
Absolute drug resistance	8	3	4	15 (26)
Relative drug resistance	17	2	0	19 (32)
Aplasia → death	2	3	1	6 (10)
Aplasia → leukemia	2	0	0	2 (3)
Aplasia → PR	4	1	1	6 (10)
Aplasia → CR	8	3	0	11 (19)

Median survival for complete responders from beginning of amsacrine therapy was 27.5 weeks (range, 7.4–86.6), with nine patients receiving other chemotherapeutic regimens after the amsacrine trial. Median survival for all evaluable patients in the study was 10.5 weeks (range, 1.8–86.6).

Toxicity

The major toxic effects included myelosuppression (100%), hyperbilirubinemia (30%), stomatitis (46%), cardiac dysrhythmia (1.5%), and alopecia (100%). The expected myelosuppression was universal, with all 29 patients who entered the study with a peripheral blood granulocyte count > 1000/μl developing agranulocytosis and all 21 patients with a pretreatment platelet count > 50,000/μl developing a platelet transfusion requirement. In patients who achieved a CR following amsacrine, the time to recovery of clinically useful granulopoiesis and thrombopoiesis after the start of the last course of chemotherapy was, on the average, between 3 and 5 weeks (table 3), not excessively long considering that most of these patients had been heavily pretreated.

Hepatic toxicity was largely confined to hyperbilirubinemia, with little evidence of hepatocellular damage. While 62% of the patients evaluable for hepatotoxicity developed some elevation in bilirubin (table 4), only 30% had grade 2 or greater toxicity (bilirubin, ≥ 3.1 mg/dl). Although the highest bilirubin level observed was 28 mg/dl, in no case did hepatic toxicity result in coma. The bilirubin elevations were transient, however, resolving in 2–4 weeks.

TABLE 3.—Recovery of granulopoiesis and thrombopoiesis after CR with amsacrine

Recovery	Median days to recovery (range)
Granulocyte regeneration	
> 500/μl	27 (21–39)
> 1000/μl	32 (21–45)
Platelet regeneration	
> 50,000/μl	30 (20–50)
> 100,000/μl	39 (20–51)
Last platelet transfusion	26 (16–40)

Stomatitis was quite variable: negligible in > 50% of the patients, but grade 2 or 3 in 46% of the patients (grade 2 = mucositis, ulcers, able to eat; grade 3 = unable to eat).

There were two episodes of supraventricular tachycardia noted during the study. One episode occurred during the first dose of amsacrine. The patient's serum potassium was not determined on the day of the dysrhythmia but was 3.2 mEq/L the day before. The second episode occurred in another patient 5 weeks after chemotherapy and was unlikely drug-related.

When evaluable, some degree of alopecia was observed universally. Nausea and vomiting, when they occurred, were usually transient and mild. There were no incidents of seizure.

In the nine patients who received amsacrine for 8 days, no differences in toxic effects, including severity of stomatitis, were noted.

DISCUSSION

This study confirms reports by others (5–7) that amsacrine is one of the most active drugs introduced in recent years for the treatment of acute leukemia. Used as a single agent, the incidence of favorable responses noted in this study is quite similar to that reported by others (5–7). This response rate compares favorably with responses seen with other active single agents when initially used for relapse reinduction. Doxorubicin, for example, was shown to induce CR in 25% of previously treated patients with ALL and in 26% of patients with AML (9). In addition, not only were the patients included in this study probably more heavily treated than those included in earlier studies with doxorubicin, but most were also anthracycline-resistant. The response rate in the 12 pa-

TABLE 4.—Hepatotoxicity with amsacrine

Bilirubin (mg/dl)	Toxicity grade	Patients with toxic effects	
		No.	%
< 1.2	0	24	38
1.2–3.0	1	20	32
3.1–7.0	2	9	14
> 7.0	3	10	16

tients with ALL was similar to that observed in AML. Similar activity of amsacrine in the treatment of ALL was observed in some (5,7) but not all (6) of the other phase II trials of amsacrine used as a single agent. Subsequent to this study, we have treated a patient with T-cell ALL and observed the acute tumor lysis syndrome following a single dose of amsacrine (10).

The toxic effects of amsacrine seen at this dose and schedule were acceptable. Myelosuppression, while severe and expected, was not prolonged, with marrow recovery apparent, on the average, between 3 and 5 weeks in the patients achieving CR. Our observation of hyperbilirubinemia in the absence of elevation in hepatic enzymes has been reported by others (5). Stomatitis, only occasionally severe with the regimen we employed, is probably the dose-limiting toxic effect for this agent in the treatment of acute leukemias (5). A particularly disturbing toxic effect, cardiac dysrhythmia (11), was seen only once during these studies. Because of a possible contributing role of hypokalemia in this problem (11), current protocols employing amsacrine require monitoring and normalization of serum potassium prior to administration of the agent. Whether such measures will eliminate the dysrhythmias remains to be demonstrated.

We and others (5–7) have given amsacrine in doses totalling 600–700 mg/m^2 without observing significant additional toxicity when compared to the 450-mg/m^2 total dose given to most patients in this study. We also observed that most patients required more than one course of therapy before acquiring an aplastic marrow or a remission. Based on the above observations, we conclude that a dose higher (600–700 mg/m^2, total dose) than we employed, administered over 5–7 days, is a better regimen for use of amsacrine as a single agent in the treatment of adult acute leukemia.

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Phase I Clinical Trial of 13-*cis*-Retinoic Acid in Myelodysplastic Syndromes[1,2,3]

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13-*cis*-Retinoic acid (13-cRA) induces maturation and differentiation of neoplastic myeloid cell lines in vitro. We conducted a phase I clinical trial of 13-cRA in patients with myelodysplastic syndromes (MDS), using a single daily oral dose schedule. Seventeen patients with MDS and one each with acute nonlymphoblastic leukemia and chronic myelogenous leukemia in blast crisis were treated with 13-cRA at doses ranging from 20 to 125 mg/m²/day. Hepatotoxicity was dose-limiting and was manifested by hyperbilirubinemia and increased SGOT levels. This effect was seen only at the highest dose level of 125 mg/m²/day and was completely reversible upon cessation of the drug. Other toxic effects were mild, and included cheilosis, hyperkeratosis, stomatitis, and elevation of serum triglyceride levels. Fifteen patients with MDS were evaluable for therapeutic response. Five patients showed improvement in hematologic parameters. These responses included normalization of bone marrow blast count and increases in leukocyte count, platelet count, and/or hemoglobin concentration. Responses were generally not seen until at least 3 weeks of therapy were completed. We conclude that further study of 13-cRA in myelodysplastic syndromes is warranted and recommend that future studies utilize a starting dose of 100 mg/m². [Cancer Treat Rep 67:981–986, 1983]

Myelodysplastic syndrome (MDS) refers to a spectrum of hematopoietic diseases which evolve into acute leukemia in approximately 40% of cases (1). Morphologic, karyotypic, and kinetic data indicate that MDS results from neoplastic transformation at a pluripotent stem cell level (2). No effective therapy is presently available for this disorder. Patients with MDS respond poorly to intensive chemotherapy, frequently developing prolonged bone marrow aplasia (3).

13-*cis*-Retinoic acid (13-cRA) is a stereoisomer of retinoic acid which is derived metabolically from vitamin A (retinol) by oxidation in the liver. Unlike vitamin A, retinoic acid is not stored in the liver; single doses are rapidly metabolized and excreted. The inability to store retinoic acid and its analogs in the liver may be advantageous, since hepatic toxicity which occurs with high concentrations of retinol may be avoided (4).

Recent in vitro studies have shown that retinoic acid inhibits tumor cell growth (5). Retinoic acid also induces differentiation in various tumor cell lines (6,7), including the human promyelocytic leukemia cell line (HI-60) (8). While the biologic effects of 13-cRA appear to be identical to those of retinoic acid, 13-cRA is four to eight times less toxic in vivo than retinoic acid in animals. Since retinoids induce terminal differentiation in neoplastic cells, it appeared reasonable that 13-cRA could be of use in the treatment of MDS, a disease characterized by a block in cell maturation.

This paper reports the results of a phase I trial in patients with MDS using a single daily oral dose schedule of 13-cRA.

PATIENTS AND METHODS

Patients with MDS were eligible for this study if they had a life expectancy > 3 months and adequate renal and hepatic function (serum creatinine and bilirubin concentration < 2.0 mg/dl). Patients had ceased prior therapy

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[3]13-*cis*-Retinoic acid was supplied by Hoffmann-La Roche Inc, Nutley, NJ.

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for this disease at least 8 weeks before entry in the study. Written informed consent was obtained from all patients and the study was approved in advance by the Clinical Investigations Committee of the Memorial Sloan-Kettering Cancer Center.

Criteria used at this center for the diagnosis of MDS have been published elsewhere (9). There were two patients entered in the study with refractory anemia and hypocellular bone marrow, 11 who had refractory anemia with "excess" (> 10%) bone marrow blasts (RAEB), and four with chronic myelomonocytic leukemia (CMMoL). One patient with chronic myelocytic leukemia in blast crisis was entered in the study after having achieved a partial response (< 25% marrow blasts) to combination chemotherapy. One additional patient with "smouldering" acute nonlymphoblastic leukemia who was clinically stable and did not require immediate intensive chemotherapy was also entered.

13-cRA was supplied in 10- and 40-mg gelatin capsules and was administered orally in a single daily dose. The initial dose of medication was maintained throughout the treatment course; dose escalation in the same patient was not permitted. Six dose levels were evaluated, ranging from 20 to 120 mg/m^2/day. An adequate trial was defined as a minimum of 21 consecutive days of therapy. In responding patients, the drug was continued until progression of disease occurred.

Patients were seen in the clinic every week for the first 4 weeks and every 2 weeks for the remainder of the treatment period. At each visit, a cbc with differential and reticulocyte counts was obtained. An SMA-12 screening profile was performed at 2-week intervals. Triglyceride levels were obtained prior to initiation of treatment and at cessation of therapy. Bone marrow aspirations were performed at initiation of treatment and at 2, 4, 8, and 12 weeks. Response to treatment was evaluated according to improvement in hematologic parameters.

RESULTS

Nineteen patients were entered in the study. The median age of patients was 62 years (range, 14–84), and the median Karnofsky performance status was 70. Twenty-five percent of patients had received prior treatment with pyridoxine and folate or with methylprednisolone, without therapeutic response. Four patients acquired MDS after chemotherapy for another malignancy. One of these four patients (No. 4) showed response to treatment with 13-cRA.

Three patients each were treated with 13-cRA at daily dose levels of 20, 45, 60, and 80 mg/m^2, respectively. Five patients received 100 mg/m^2/day and two patients were treated with 125 mg/m^2/day. Our dose escalations represent a modification of the Fibonacci scale. Toxic effects necessitating withdrawal of drug were seen in two of two patients at the 125-mg/m^2 dose, while no dose-limiting toxic effects were observed at the 100-mg/m^2 dose.

Hepatotoxicity was dose-limiting and was manifested by hyperbilirubinemia (> 3.5 g/dl) and increased SGOT levels (> 350 IU). This effect occurred in both patients receiving 125 mg/m^2 and became evident within 3 weeks of initiation of therapy. Evidence of hepatotoxicity resolved completely within 10 days after discontinuation of the drug. The most common toxic effects of 13-cRA were hyperkeratosis and cheilosis. However, the hyperkeratosis was mild and easily alleviated by application of a urea-based skin cream. Other manifestations of drug toxicity are summarized in table 1. Mild nausea and/or anorexia was seen in two of the patients. Mild hypertriglyceridemia (> 25% increase from baseline levels) was seen in four patients at dose levels > 80 mg/m^2.

Fifteen of 17 patients with MDS were evaluable for therapeutic response. The inevaluable patients included one with RAEB who died of progressive disease after 13 days of therapy, and another patient with refractory

TABLE 1.—Toxic effects observed in patients treated with 13-cRA*

	Dose level (mg/m^2/day)					
Toxic effect	20	45	60	80	100	125
Cheilosis	3	2	4	3	5	2
Hyperkeratosis	3	2	4	3	5	2
Stomatitis		1	1	1	2	1
Nausea					1	1
Serum triglyceride concentration						
25% increase from baseline				1	2	1
50% increase from baseline						1
Hepatotoxic effects						
(50% increase from baseline)						
SGOT						2
Bilirubin						2

* Values = No. of patients.

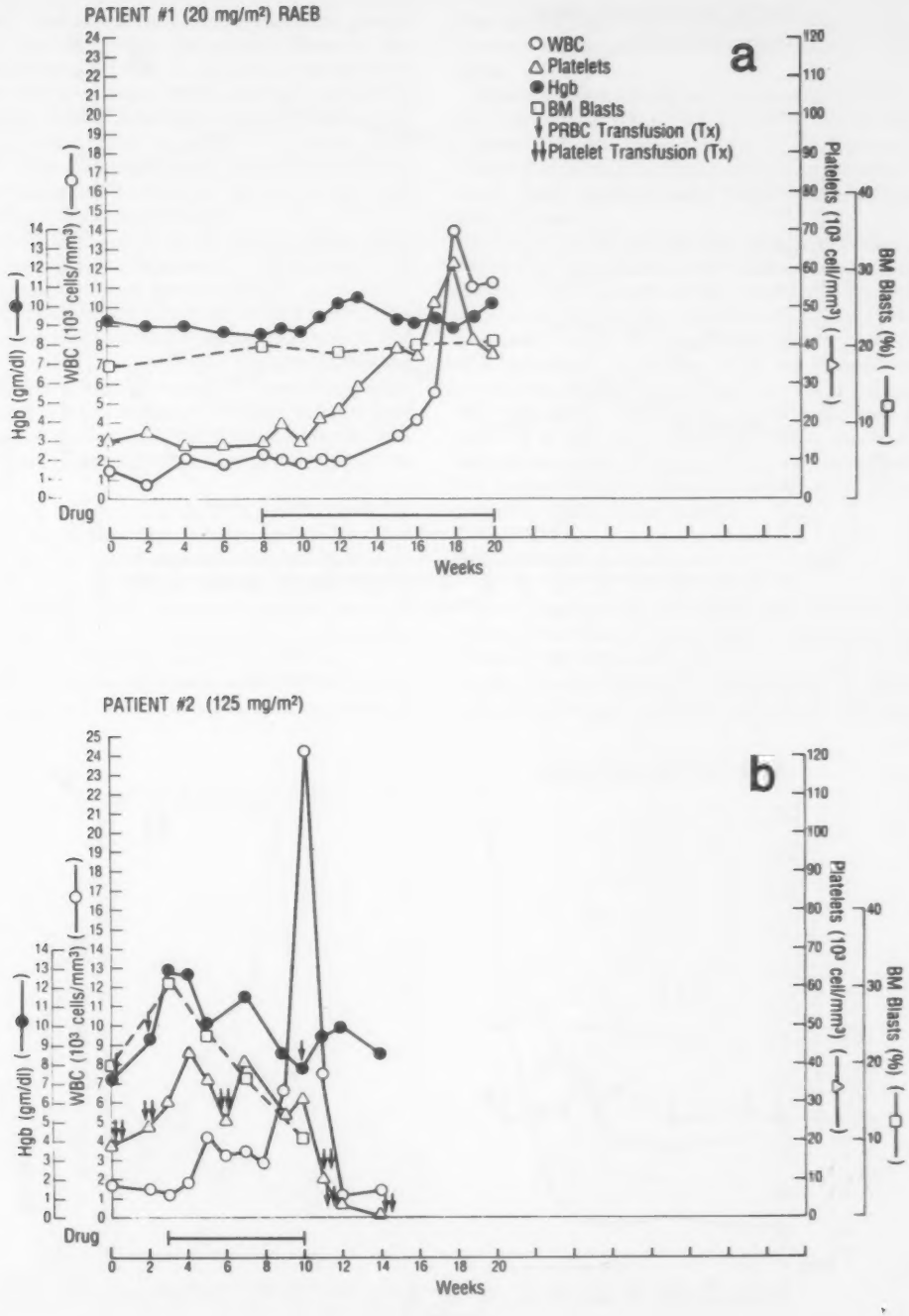

FIGURE 1.—Hematologic response of 2 patients with RAEB to 13-cRA. a: Patient 1. b: Patient 2.

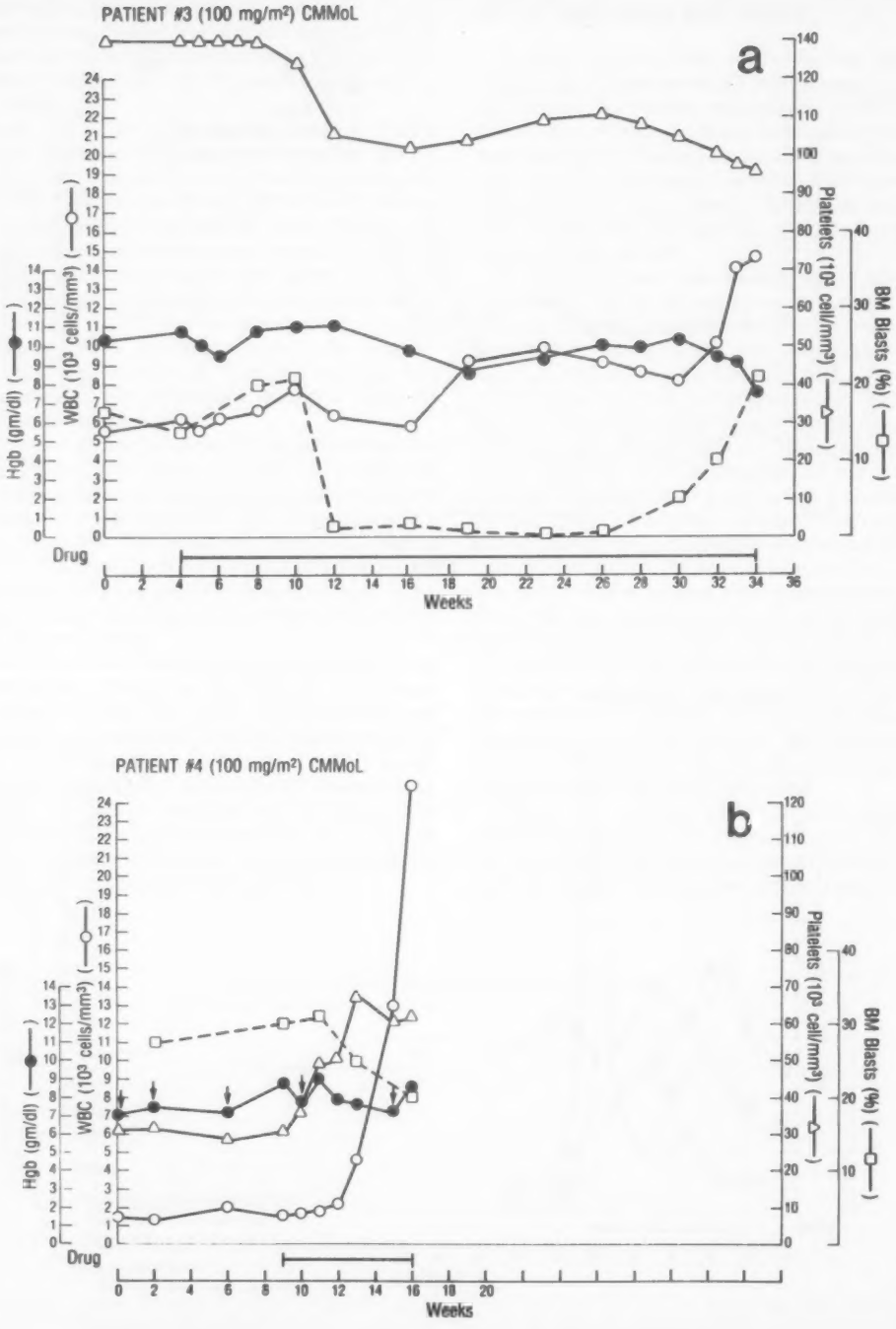

FIGURE 2.—Hematologic response of 2 patients with CMMoL to 13-cRA. a: Patient 3. b: Patient 4.

anemia who had normalization of hematologic parameters after starting 13-cRA (100 mg/m²). However, this patient inadvertently failed to discontinue therapy with androgens and prednisone, which had been started 13 weeks earlier without detectable response. The remaining two patients (chronic myelocytic leukemia in blast crisis and acute nonlymphoblastic leukemia) developed rapid deterioration of hematologic parameters and were treated with cytotoxic chemotherapy.

Of the 15 evaluable patients, five experienced hematologic responses. Responses were not generally seen until at least 3 weeks of therapy were completed.

Patient 1, a 76-year-old man with RAEB treated at the 20-mg/m² dose level, showed a marked increase in wbc and platelet counts for a period of 6 weeks before becoming ill with an intercurrent medical illness (congestive heart failure) (fig 1a). However, the bone marrow blast count remained abnormal (> 5%). It was noteworthy that the majority of neutrophils seen showed Pelger-Huet abnormalities.

Patient 2, a 72-year-old male with RAEB, showed a marked increase in wbc count and a decrease in blast percentage at the 125-mg/m² dose level (fig 1b). In this patient, 13-cRA was discontinued because of toxic effects. A prompt decrease of wbc and platelet counts was noted upon cessation of therapy. Unfortunately, this patient refused further treatment and was lost to follow-up.

Patient 3, a 52-year-old female with CMMoL treated with 100 mg/m² of 13-cRA, showed a normalization of bone marrow blast count for a period of 20 weeks (fig 2a). However, the disease eventually progressed to acute leukemia.

Patient 4 was a 48-year-old woman with CMMoL who was treated with 100 mg/m² of 13-cRA. She showed an increase in wbc and platelet counts (fig 2b). However, this patient died of an infection in spite of an increased wbc count. Again, predominantly Pelger-Huet granulocytes were observed.

Patient 5, a 38-year-old man with RAEB which occurred after chemotherapy for Hodgkin's disease, experienced a marked increase in wbc count and a concomitant decrease in transfusion requirements at the 45-mg/m² dose level. During 13-cRA therapy, this patient developed salmonella septicemia while on therapy, which proved very difficult to eradicate, in spite of an elevated wbc count (fig 3). Pelger-Huet abnormalities were again observed in the neutrophil series. These abnormalities were not seen prior to therapy in any responding patient. The patient was subsequently treated with high-dose cytarabine and died due to sepsis.

DISCUSSION

The biologic activity of retinoic acid parallels that of retinol, except that it cannot replace vitamin A in the formation of visual pigments in the maintenance of normal reproductive processes (4). The mechanism of action of retinoic acid is not completely understood. Vitamin A is

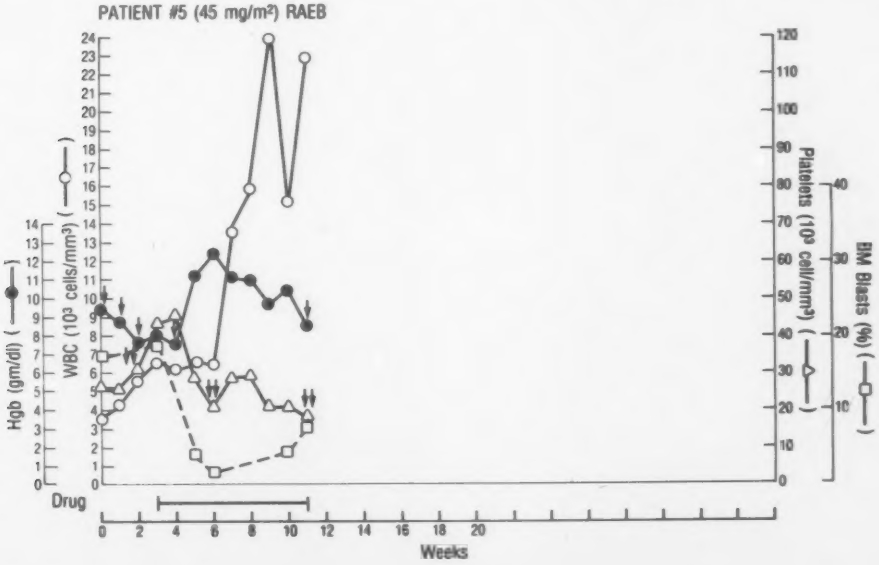

FIGURE 3.—Hematologic response of Patient 5 with RAEB to 13-cRA.

involved in cellular differentiation and the proper maintenance of epithelia (10). Feeding animals high doses of retinoids after carcinogen exposure reduces the incidence of neoplastic changes (11). In addition, retinoic acid inhibits the growth of certain tumors in vitro (12). As previously noted, retinoic acid causes terminal differentiation in several cell lines, including the human promyelocytic leukemia cell line (Hl-60).

In this trial, patients with MDS were selected because of the possibility of preventing progression to overt leukemia and because no other effective treatment is currently available. Since MDS responds poorly to cytotoxic chemotherapy, these patients are generally treated supportively (3).

We found that 13-cRA had acceptable toxicity and could be safely administered as a single daily dose of 100 mg/m^2. Hepatotoxicity was dose-limiting. All toxic effects were completely reversible upon discontinuation of the drug. Other dose schedules, such as multiple daily administration, were not investigated in this trial.

Since patients with MDS who do not develop leukemia generally succumb to the complications of progressive pancytopenia (1), the evidence of therapeutic activity obtained in this trial is notable. However, our results are preliminary and further investigation is needed. A randomized clinical trial of 13-cRA is indicated to evaluate the clinical efficacy of this agent in patients with MDS. Such a trial has recently been initiated at our institution. Further studies investigating other dose schedules, such as daily multiple repeated doses or low-dose continuous therapy, as well as combinations with other agents, are also warranted.

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Phase I Investigation of Ametantrone[1,2]

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Ametantrone is the third of a family of anthracene derivatives to undergo a phase I trial in man. Sixteen patients received 33 courses of drug as a single iv dose given every 3 weeks. Escalations proceeded from 120 to 180 mg/m². Predictable and reversible leukopenia was the dose-limiting toxic effect. Four patients developed thrombocytopenia. Nonhematologic toxic effects included a marked cumulative blue discoloration of the skin seen in all patients receiving more than three courses of the drug. This cumulative cosmetic effect may also be dose-limiting. Other nonhematologic toxic effects included: blue urine (all patients), nausea (two), vomiting (one), a blue stool (one), and reversible elevations of either SGOT or alkaline phosphatase (two). No objective responses were seen in this study. A dose of 140–160 mg/m² is recommended as the starting dose for phase II trials in patients who have received prior chemotherapy or radiotherapy. [Cancer Treat Rep 67:987–991, 1983]

Ametantrone is a new anthracene derivative. This dark blue compound is the parent compound in a family of anthracene derivatives currently in clinical trials. Two other anthracene compounds, mitoxantrone and bisantrene, have completed phase I testing and are currently in phase II trials.

Ametantrone was selected for phase I testing because it had significant antitumor activity in a number of animal tumor systems including L1210 and P388 leukemias; B16 melanoma; colon tumors 26, 36, 38, 06A, and 07A; ovarian carcinoma M5076; and CD8F1 mammary adenocarcinoma. It also has moderate activity against subrenal capsular implants of the following human tumors: colon CX-1, colon CX-5, mammary MX-1, and lung LX-1 (1).[6]

Animal toxicology for ametantrone was investigated in dogs and mice. In dogs, the principal target organs for toxicity were bone marrow, lymphoid tissue, gastrointestinal tract, and testes. Hematopoietic side effects were dose-related reversible leukopenia, granulocytopenia, and thrombocytopenia. In addition, blue urine, blue stools, emesis, intermittent blood-tinged diarrhea, anorexia, weight loss, hypothermia, lameness, and conjunctivitis were noted. At high doses, blood biochemical changes suggestive of hepatic and renal dysfunction were present, with congestion and hemorrhage in the gastrointestinal tract. The toxic dose low in dogs was 13.6 mg/m².[6]

Ametantrone was tested for cardiotoxicity in the rat model of Zbinden and in the New Zealand White rabbit. In the rat model, treated in parallel with equitoxic doses of doxorubicin and daunorubicin, ametantrone showed a cumulative dose-related cardiac toxicity. In rabbits randomized to be treated with either doxorubicin at 2 mg/kg/week or ametantrone at 4 mg/kg/week, all 12 doxorubicin-tested animals died by 15 weeks, whereas only three of the ametantrone animals were dead at 15 weeks. Histologic examination of the animals showed moderate to severe myocardial injury in most of the doxorubicin-treated rabbits, with less severe and less frequent lesions noted in the ametantrone-treated group.[6]

A recent phase I study of ametantrone was conducted by Piccart et al (2) in 19 patients with solid tumor, using a single iv dose of drug ranging from 10 to 180 mg/m² administered every 3 weeks. The major dose-

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[5]Pharmaceutical Research Division, Warner-Lambert Co, Ann Arbor, MI.

[6]Summary for Investigators—on Ametantrone Acetate (USAN) (CI-881), (NSC 287513) by the Warner-Lambert/Parke-Davis Co, Pharmaceutical Research Division, Ann Arbor, MI, Jan 21, 1981.

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related and dose-limiting side effect was a rapidly reversible leukopenia. Only two patients had platelet counts < 100,000/μl. Other side effects were reversible blue skin discoloration (five patients), blue urine for up to 72 hours after receiving the drug at doses > 40 mg/m² (three), transient orthostatic hypotension (three), minor stomatitis (one), minor hair loss (three) and nonspecific ECG changes (two). The dose suggested by these authors for starting phase II trials in patients with prior exposure to chemotherapy was 135 mg/m² given as a single iv dose every 2-3 weeks.

The present limited phase I trial was performed to gain experience with this new anthracene derivative prior to anticipated disease-oriented phase II trials with the drug.

MATERIALS AND METHODS

Patient Selection

All patients who were entered in this trial had a histologically confirmed diagnosis of cancer (table 1). Only patients who had an estimated survival of 8 weeks were eligible. All but two patients had received prior treatment with multiple chemotherapeutic agents (an average of three different drugs), and 64% had received prior radiotherapy to the primary tumor or metastatic site. All patients had measurable disease at the time of entry in this study.

Prior to therapy, all patients had adequate blood cell counts (total wbc count ⩾ 3500/μl and platelet count ⩾ 150,000/μl). All patients had to have alkaline phosphatase < 150 IU/ml, bilirubin < 1.5 mg/dl, creatinine < 2.0 mg/dl, and BUN < 20 mg/ml. Prior to entry, written informed consent was obtained from the patient in accordance with federal and institutional policies.

TABLE 1.—Characteristics of patients in phase I study of ametantrone

Characteristic	No. of patients
Total in study	16
Total evaluable for toxic effects	16
Males	16
Females	0
Age (yrs)	
Median: 59	
Range: 46-69	
Performance status*	
Median: 1	
Range: 0-3	
Primary tumors	
Lung (small cell and non-small cell) carcinoma	6
Head and neck squamous cell carcinoma	4
Colon carcinoma	2
Renal cell carcinoma	2
Melanoma	1
Duodenal (adenocarcinoma)	1
Previous treatment	
None	1
Chemotherapy only	5
Radiotherapy only	1
Chemotherapy and radiotherapy	9

* Southwest Oncology Group criteria.

Treatment Plan

Ametantrone for clinical use was supplied by the Warner-Lambert Co, Ann Arbor, MI, as a sterile solution for injection in 10-ml ampules containing 100 mg of ametantrone as a diacetate salt. At the start of the study, the appropriate dose of ametantrone was removed from the ampule and was further diluted with 100 ml of 5% dextrose in water. The resulting dark blue solution was infused over 30 minutes through a newly started, well-running iv line. The schedule of drug administration was a single dose every 21 days.

Based on the conclusion by Piccart et al (2) that 135 mg/m² was a reasonable dose to commence phase II trials in pretreated patients, 120 mg/m² was chosen as the starting dose for this limited phase I trial. Dose levels were increased by 20 mg/m² as noted in table 2, and except for the 180-mg/m² dose, a minimum of three patients were treated at each dose level. If no toxicity was observed at a particular dose level after 4 weeks, the new dose level was started. Dose escalations were performed in some patients. These escalations were performed only after it was certain that the higher dose was tolerable in at least two patients not previously treated with ametantrone. After escalation to 180 mg/m², additional patients were selected for the 140-mg/m² and 160-mg/m² levels in an attempt to secure a starting dose for phase II trials.

Study Parameters

During treatment the patients had cbcs with differential and platelet counts biweekly. Chemistry panels and urinalysis were done once per week. Every 3 weeks, the patients had prothrombin time, partial thromboplastin time, ECG, and other radiologic or laboratory examinations pertinent to tumor evaluations. Patients were followed closely for evidence of toxic effects and for other biologic effects. Standard criteria of response were used to evaluate the antitumor effect of the drug. These criteria included: (*a*) complete remission—disappearance of all clinical evidence of active tumor and tumor-related symptoms for at least 4 weeks; (*b*) partial remission— ⩾ 50% decrease in the sum of the product of the perpendicular diameters of all measured lesions for at least 4 weeks; (*c*) no change—objective tumor regression not qualifying for partial remission but lasting at least 4 weeks, or a steady state not qualifying for increasing disease for at least 8 weeks; and (*d*) increasing disease—unequivocal

TABLE 2.—Myelosuppression by ametantrone at each dose level

Dose (mg/m^2)	No. of patients	No. of patients without prior ametantrone*	No. of courses	Median nadir (range), × 10^3 cells/μl		
				Wbcs	Granulocytes	Platelets
120	4	4	1	3.6 (2.1-4.7)	2.1 (1.1-2.9)	361 (225-602)
140	10	8	23	2.4 (0.2-3.9)†	1.6 (0.06-3.7)†	166 (16-433)†
160	4	3	8	2.0 (0.9-2.4)‡	1.2 (0.7-2.8)‡	238 (147-313)‡
180	1	1	1	1.5	0.6	129

*No. of patients who had not received ametantrone at previous dose levels.
†Includes a patient with pancytopenia secondary to sulfa, and a patient with a right upper lobe lung abscess.
‡Includes a patient with superior and inferior vena cava syndrome receiving steroids to decrease edema.

increase of at least 25% in the size of any measured lesion or the appearance of any new lesion.

In view of the report of the cardiotoxicity of mitoxantrone reported by Yap et al (3), some patients receiving ⩾ 140 mg/m^2 of ametantrone underwent radioisotopic ejection fraction and wall motion studies before infusion, at 24 hours after infusion, and at 3-week posttreatment follow-up, in an effort to detect any early or late drug-induced cardiac toxicity. The same group of patients had Holter monitoring prior to, during, and for 2 hours after infusion, in an attempt to detect any cardiac rhythm abnormalities.

Termination of Study

Individual patients were taken off study if objective tumor progression occurred following one or more courses of ametantrone. The phase I study was terminated when the maximally tolerated dose was established.

RESULTS

Over a 9-month period, 16 patients were entered in the study. The characteristics of these patients are detailed in table 1. Their ages ranged from 46 to 69 years (median, 59). All patients were evaluable for toxic effects. The sequence of doses administered is detailed in table 2. Thirty-three courses of the drug were administered. Eight patients had one course, four patients had two courses, two patients had three courses, one patient had four courses, and one patient had seven courses of ametantrone.

Myelosuppressive Toxicity

Leukopenia was the dose-limiting toxic effect of ametantrone (table 2). As noted in table 2, the degree of leukopenia and granulocytopenia was related to the dose of ametantrone administered. The median time to occurrence of the wbc nadir was 12-13 days, with full recovery to baseline pretreatment values by Days 18-19. Two patients at the 140-mg/m^2 level, one receiving concomitant radiotherapy for brain metastases and one receiving concomitant trimethoprim-sulfamethoxazole because of an indwelling Foley catheter, had delayed recovery to baseline values. Otherwise, a dose of 140-160 mg/m^2 provided reproducible and reversible leukopenia and granulocytopenia in patients previously treated with radiation and/or chemotherapy. The one patient receiving the 180-mg/m^2 dose developed leukopenia that was severe enough to preclude further patient entry at that dose level.

The degree of thrombocytopenia was mild and appeared unrelated to the dose of ametantrone administered. Anemia was induced by the administration of ametantrone in the one patient who had been receiving the trimethoprim-sulfamethoxazole. The degree (if any) of cumulative myelosuppression induced by ametantrone could not be assessed because too few patients received repeated courses of drug at the maximally tolerated doses.

Nonmyelosuppressive Toxicity

All light-complected patients receiving two or more courses of ametantrone developed a cumulative blue-gray skin discoloration. Two of four patients receiving three courses or more of ametantrone also complained of dry skin with pruritis. After the drug was stopped, a peeling of the epidermis occurred along with a gradual fading of the blue-gray skin tint over the ensuing months. However, even 5 months after discontinuation of the drug, two of these patients still have a blue-gray discoloration of their skin. Attempts were made to locate the drug in the dermis and epidermis. A patient who had received four courses of ametantrone had 5-mm punch biopsy specimens of skin obtained for pathologic analysis to determine the location of the drug in the skin. Part of the biopsy specimen was quick-frozen, while the other part was processed routinely for paraffin infiltration and hematoxylin-eosin staining. The frozen sections were observed directly, without staining, with a regular light and a fluorescent (390-440-nm band width) microscope. Despite such techniques, no drug could be demonstrated in the biopsy specimens.

Two patients complained of dizziness or light-headed-

ness 1 week after infusions of ametantrone at 120 and 160 mg/m^2. On examination, the patients had no demonstrable orthostatic change in blood pressure. Two patients had nausea and one had vomiting with infusion of the drug. Loose stools were seen in two patients (but no increase in stool frequency was noted). Blue urine occurred in all patients up to 48 hours after the infusion of the drug. A blue stool was documented in one patient during administration of the drug. Reversible mild increases (< 50%) in SGOT or alkaline phosphatase were noted in two patients.

Four patients, at ametantrone doses ⩾ 140 mg/m^2, had Holter monitoring before, during, and after the infusion of drug. Two patients were found to have rare baseline premature ventricular contractions (six to seven/hour) that did not increase with administration of ametantrone. A third patient had T-wave inversion before receiving the drug, and this continued through the drug infusion with resolution shortly after completion of the infusion. A fourth patient had nonspecific ST-T wave changes as a baseline. These changes did not increase with administration of ametantrone.

As an additional part of the protocol, four patients, at ametantrone doses ⩾ 140 mg/m^2 had radioisotopic ejection fraction and wall motion studies (MUGA scans) before treatment and 24 hours after treatment, with another follow-up study at 3 weeks after treatment, in an attempt to document any decrease in ejection fraction caused by the drug. No significant change from baseline was noted in the right and left ventricular ejection fractions in the four patients receiving the scans 24 hours after infusion of ametantrone. The three patients receiving MUGA scans 3 weeks after treatment demonstrated no decrease in ventricular ejection fractions. Two other patients placed on study before the cardiac monitoring had MUGA scans done after their third and fifth treatments, respectively, with normal right and left ejection fractions noted in each patient. No patient developed congestive heart failure while on study.

Antitumor Activity

No objective tumor responses were noted in this trial. One patient with colon cancer and lung metastases had stable disease over 4 months, and another patient with renal cell cancer and metastatic disease to the lumbar spine had stable disease for 3 months. One patient with squamous cell carcinoma of the head and neck with extensive cutaneous metastases had a mixed response after a single course of the drug. He also had marked relief of pain associated with cutaneous nodules. After two more courses of ametantrone, the patient's responding nodules began to increase in size and he was withdrawn from the study.

DISCUSSION

Ametantrone is the third anthracene derivative undergoing clinical trials for the treatment of human cancer. Like mitroxantrone and bisantrene, ametantrone has predictable and rapidly reversible leukopenia as a dose-limiting toxic effect when given as a single iv dose every 3 weeks. Unlike the other anthracene derivatives, ametantrone demonstrates a cumulative blue-gray skin discoloration that is most noticeable in light-complected patients. The blue-gray discoloration has not been entirely reversible for as long as 5 months after stopping the drug.

The phase I trial by Piccart et al (2) proposed a dose of 135 mg/m^2 for phase II trials in previously treated patients with solid tumor. The present abbreviated phase I trial demonstrated predictable and reversible leukopenia at the 140–160-mg/m^2 dose levels. The observed wbc, granulocyte, and platelet counts at these levels are not markedly different. Skin discoloration after three courses of ametantrone is also a cosmetically dose-limiting side effect. Overall, a dose of 140–160 mg/m^2 can be recommended as a phase II starting dose for patients with heavy prior treatment.

One major question in future clinical testing of ametantrone will be whether it possesses any advantages (or disadvantages) over its analog mitoxantrone. In initial clinical trials, mitoxantrone has had documented antitumor activity against breast cancer (3,4) and acute leukemia (5), but it has been inactive against colorectal cancer (6). Phase II studies with ametantrone will have to be performed in patients with refractory breast cancer and acute leukemia not previously exposed to mitoxantrone. If antitumor activity is noted in patients with those malignancies, then a head-to-head comparison between ametantrone and mitoxantrone should be performed to define which compound is superior in terms of response rate and/or toxicity. The activity of ametantrone in numerous mouse colon tumors and human colon cancer xenografts provides an important lead for phase II trials with the drug. Since mitoxantrone has already proven inactive in patients with previously untreated colon cancer, additional comparative trials between ametantrone and mitoxantrone in patients with colon cancer are unnecessary.

Overall, clinical comparisons of these two anthracene derivatives should provide important feedback to analog programs attempting to find an anthracene derivative with improved antitumor activity.

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Phase II Study of Aziridinylbenzoquinone in Refractory Lymphoma[1,2]

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Aziridinylbenzoquinone (AZQ), an alkylating agent with lipophilic properties allowing CNS penetration, has been studied in a phase II trial in patients with refractory lymphoma. Thirty-five patients (ages, 24–84 years) have been treated using AZQ at a dose of 30 mg/m² every 3 weeks. Nine patients (26%) have responded (complete responses for 14+, 12+, 4, 3, and 3 months; partial responses for 7, 2, 2, and 2 months). Seven of the nine responses were seen in patients with diffuse histologies, particularly diffuse histiocytic lymphoma. One of two patients with CNS lymphoma responded completely. Complete remissions were observed only in patients receiving not more than one prior chemotherapeutic protocol. The drug was well-tolerated. Neutropenia was brief and acceptable. However, cumulative thrombocytopenia occurred even in patients without prior nitrosourea therapy and necessitated significant dosage reductions with subsequent cycles. AZQ appears active in lymphomas, especially in diffuse histiocytic lymphoma. The possibility of using AZQ in CNS lymphoma should be further explored. [Cancer Treat Rep 67:993–996, 1983]

Aziridinylbenzoquinone (AZQ) represents a rationally synthesized drug in which the molecular characteristics of good lipid solubility, low ionization (to penetrate the CNS), and good water solubility are optimized (1–4). AZQ appears to act as an alkylating agent (1). Phase I studies in solid tumors revealed that the dose-limiting toxic effects were leukopenia and thrombocytopenia (5,6).[4] Several phase II trials in solid tumors have been reported, with few responses seen in lung cancer (7,8), genitourinary cancer (9), or colorectal disease (10,11). However, encouraging results have been seen in recurrent primary and metastatic brain tumors (12,13). Cumulative thrombocytopenia has been noted, particularly after prior nitrosourea therapy (9,12,13).

A trial of AZQ in refractory lymphoma has previously been reported with only two minor responses observed in 20 patients.[5] The following report represents a phase II trial using AZQ at a dose of 30 mg/m² every 3 weeks to assess response rate and duration in patients with refractory lymphomas.

MATERIALS AND METHODS

Patients with lymphoproliferative disorders who had received prior chemotherapy with at least one combination chemotherapy regimen and who had relapsed or failed to respond were eligible for this study. Prior to initiating therapy, informed consent was obtained according to institutional guidelines.

Before starting therapy, all patients had a complete history and physical examination, and underwent additional procedures as needed to define the extent of disease, including chest x-ray, abdominal sonogram and/or computerized tomographic scan, and bone marrow aspiration with biopsy. Pretreatment laboratory tests included cbc with platelet count, liver and renal function tests, coagulation profile, and electrolytes. During therapy, patients had follow-up laboratory studies, including cbc with platelet count, liver and renal function tests, coagulation studies, and electrolytes on Days 7, 10, and 14 of the first cycle and prior to each subsequent

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[4]Minutes of Phase I Working Group. National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD, July 16, 1980.

[5]Minutes of phase II meeting. Investigational Drug Branch, NCI, NIH, Bethesda, MD, Dec 1981.

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cycle. X-ray and bone marrow studies were repeated before each cycle, depending on initial involvement with disease. Histopathologic review of outside material was obtained for every patient, utilizing the Rappaport classification (14).

Complete remission (CR) and partial remission (PR) were defined utilizing standard criteria (15). Patients achieving less than a PR or having stable progressive disease were considered as failures on treatment. Patients dying of progressive disease before completing one cycle of treatment were also considered failures.

AZQ was supplied by the Investigational Drug Branch, Division of Cancer Treatment, NCI, Bethesda, MD. Each prescribed dose of AZQ was added to 100 ml of normal saline and infused over 30 minutes. A dose of 30 mg/m^2 of AZQ every 3 weeks was utilized. Therapy was continued in responders as scheduled until relapse or for 1 year if a CR was achieved. The dose of AZQ was reduced by one-half if the pretreatment wbc count was $< 3000/mm^3$ and/or the platelet count was $< 100{,}000/mm^3$.

RESULTS

Response to Therapy

Thirty-five patients with refractory lymphomas ranging in age from 24 to 84 years (median, 66) have been treated (table 1). One-half of the patients had a performance status of ⩾ 70 (Karnofsky scale). The majority of patients had diffuse histiocytic lymphoma or nodular poorly differentiated lymphocytic lymphoma. All except one of the patients received prior chemotherapy, 60% of whom received an anthracycline and 40% a nitrosourea. Nine patients have responded to AZQ. In patients with non-Hodgkin's lymphoma, responses were seen almost exclusively in patients with diffuse histologies. Of these 22 patients, there were seven responses, four of which were complete. In patients with nodular lymphoma (8), there was only one PR of brief duration. No patient who failed to respond by two cycles responded with further treatment. The median number of cycles of AZQ administered was three (range, one to 12). The difference in

TABLE 1.—Responses to AZQ in 35 patients with lymphoma

Histology (No. of patients)	Initial treatment protocol*	Duration of CR in mos (sites of disease)	Duration of PR in mos (sites of disease)
Diffuse histiocytic lymphoma (16)	M-BACOD (ref 15) or CHOP-bleomycin	14+ (nodes),† 3 (CNS and lung)†	7 (nodes),† 2 (nodes and skin),† 2 (nodes)†
Nodular poorly differentiated lymphocytic lymphoma (8)	M-2 (ref 16) or COP	—	2 (nodes)
Diffuse poorly differentiated lymphocytic lymphoma (3)	M-2	3 (nodes)†	—
Diffuse mixed histiocytic-lymphocytic lymphoma (1)	M-2	4 (nodes and bone marrow)†	—
Hodgkin's disease (3)	MOPP/ABDV (ref 17)	12+ (skin)†	—
Diffuse well-differentiated lymphocytic lymphoma (2)	M-2	—	—
Lymphoblastic lymphoma (2)	APO (ref 18)	—	—

*M-BACOD = methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone; CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; M-2 = carmustine, cyclophosphamide, vincristine, melphalan, and prednisone; COP = cyclophosphamide, vincristine, and prednisone; MOPP = mechlorethamine, vincristine, prednisone, and procarbazine; ABDV = doxorubicin, bleomycin, dacarbazine, and vinblastine; APO = doxorubicin, prednisone, and vincristine.

†Designates patients treated in 1st relapse.

response between the nodular and diffuse lymphomas may be due at least in part to the amount of prior therapy. Patients with nodular lymphomas received two or more prior regimens of chemotherapy, compared with one regimen for the patients with diffuse histologies. Two patients with lymphoblastic lymphoma (T-cell) and three patients with Hodgkin's disease were treated. In this group, one patient with biopsy-proven skin recurrence of Hodgkin's disease has responded completely with a current duration of remission of 12+ months.

Two patients with diffuse histiocytic lymphoma having CNS disease with mass lesions were treated with AZQ. One patient with biopsy-proven CNS disease (and lung involvement) responded completely in both sites. This patient was originally treated for primary CNS lymphoma with radiotherapy. At the time of relapse, additional radiotherapy was not considered because of tissue necrosis, and systemic high-dose methotrexate was not utilized because of a reduced creatinine clearance.

Toxicity

AZQ was well-tolerated. Nausea and vomiting was seen in only four patients, and alopecia developed in one patient. One patient had a febrile reaction after the first dose of drug, but not with subsequent doses. Renal and liver function abnormalities were not seen.

The major toxic effect was myelosuppression. The median wbc count nadir was 2300/mm^3 (range, 300–10,500/mm^3) and the platelet count nadir was 80,000/mm^3 (range, 3000–262,000/mm^3). The nadir for leukopenia and thrombocytopenia occurred 10 days after therapy (range, 7–14 days). The duration of myelosuppression was brief, with recovery occurring within 3–7 days. Severe myelosuppression occurred in only two patients, each with lymphoblastic lymphoma with bone marrow involvement.

Cumulative thrombocytopenia occurred and was independent of prior nitrosourea therapy. The fall of the median nadir platelet count related to cycles of AZQ therapy is depicted in figure 1. By the fifth cycle of AZQ, the median nadir platelet count was 50,000/mm^3. Fifty percent of patients receiving two to five cycles of AZQ required attenuation of the dose of AZQ because of progressive thrombocytopenia, despite an adequate granulocyte count. Both patients receiving 12 cycles of therapy have required 3 months to recover to a platelet count of > 150,000/mm^3 after treatment was discontinued. There was no significant cumulative effect on the wbc count with successive courses of AZQ.

DISCUSSION

In this phase II study, AZQ, a synthetic benzoquinone with apparent alkylating agent activity, was used to treat patients with refractory lymphoma. Of 35 patients treated with 30 mg/m^2 of AZQ every 3 weeks, nine have responded (five CR and four PR). Most responses were seen in patients with diffuse lymphomas. Only one of eight patients with nodular lymphomas responded.

Responses were dependent upon extent of prior treatment. CR were seen only in patients receiving no more than one prior combination chemotherapeutic program. In general, the patients with diffuse lymphoma received only one prior program. Patients with nodular lymphomas had more indolent courses and were more frequently treated at the time of first relapse with the original or other conventional programs. The difference in the response rate in this series compared with the other phase II study of AZQ in lymphoma[5] is probably explained by the consideration of treatment of first-relapse patients with AZQ. Each of these patients had relapsed within 3 months to 2 years after an initial multidrug regimen. The limited response rate in patients receiving two or more regimens of therapy before AZQ suggests considerable cross-resistance with the prior alkylating agents utilized.

The CR in one of two patients with solid CNS lesions is consistent with the observations in the phase II brain tumor series (12,13), in which therapeutic levels of AZQ are achievable in the CNS because of the lipophilic properties of the drug. Cerebrospinal levels of AZQ have been found to be two-thirds of the plasma levels. AZQ may merit further study in the systemic therapy of CNS lymphoma.

The cumulative thrombocytopenia noted in this series may complicate the application of AZQ. Dose reductions in one-half of the patients receiving more than two cycles of AZQ may have affected the type or duration of remissions achieved. The duration of thrombocytopenia in patients receiving extended therapy with AZQ may compromise the combination of AZQ with other drugs, as well as adequate dose schedules. In the present series, prior nitrosourea therapy did not account for the cumulative effect on the platelet count.

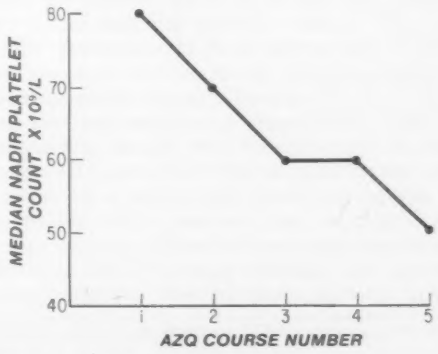

FIGURE 1.—Change in median nadir platelet counts with successive courses of AZQ.

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Phase II Study of JM8, a New Platinum Analog, in Advanced Ovarian Carcinoma[1]

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A phase II study of JM8, an analog of cisplatin, has been carried out in 36 patients with advanced ovarian carcinoma. Thirty-three of these patients had recurrent carcinoma and had been heavily pretreated, either with cytotoxic drugs alone (28 patients) or with cytotoxic drugs and radiotherapy (five patients). All patients had previously received cisplatin, either alone or as part of a combination, and some of them had previously been treated with as many as four different chemotherapy regimens. In addition, three patients with stage III ovarian carcinoma were treated with JM8 as first-line therapy. Twenty-eight of the 33 pretreated patients were evaluable for response, and five partial and two complete remissions were achieved. Of the seven responders, four had previously been shown to be completely resistant to cisplatin, and a fifth had initially responded to, but subsequently relapsed, while receiving cisplatin. Renal, aural, and neurotoxic effects were less than would have been expected with cisplatin, but hematologic toxic effects were common, with a wbc count of $< 2000 \times 10^9$/L recorded in six patients, and a platelet count of $< 50{,}000 \times 10^9$/L recorded in eight patients. [Cancer Treat Rep 67:997–1000, 1983]

The activity of cisplatin (cis-diammine-dichloroplatinum II) in ovarian carcinoma was first reported by Wiltshaw and Carr (1) in 1974, and it is now recognized as one of the most active agents for FIGO stage III and IV disease (2,3). Unfortunately, however, it is also an extremely toxic drug, causing emesis, renal impairment, peripheral neuropathy, high-frequency hearing loss, and anemia (4). As a result of these serious toxic effects, the Institute of Cancer Research, in collaboration with Johnson Matthey Research, has looked at many platinum complexes with a view to finding one which was less toxic than, but at least as active against tumors, as the parent compound. JM8 (cis-diammine I,I cyclobutane dicarboxylate platinum II) was chosen for further clinical study as a result of extensive preclinical investigations (5), and a phase I study has shown that the major dose-limiting toxic effect in man is myelosuppression, the nadir being reached about 3 weeks after treatment (6). Previous myelosuppressive therapy increased the possibility of this complication.

We report here our initial experience with a phase II study of JM8 in the treatment of patients with advanced ovarian carcinoma. Not only was the activity of JM8 in ovarian carcinoma examined, but evidence of tumor cross-resistance between JM8 and cisplatin was looked for. We have also examined the toxicity of JM8 in those tissues normally damaged by cisplatin.

PATIENTS AND METHODS

Patients

Between March 1981 and March 1982, 36 patients suffering from histologically proven advanced ovarian carcinoma, who had a World Health Organization (WHO) performance status of at least two, were entered in this study and received a total of 142 courses of JM8. Thirty-three of these patients were suffering from recurrent ovarian cancer, following treatment with conventional therapy. Twenty-eight of these patients had been treated with chemotherapy alone, and five had been treated with chemotherapy and radiotherapy. All patients had received cisplatin either alone or as part of a combination. Twenty-six patients had been treated with at least two, and four patients with as many as four, different chemotherapy regimens. In addition to the 33 patients suffering from recurrent ovarian carcinoma, three previously untreated patients

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[4]We are grateful to Bristol Myers, Johnson Matthey Research, and the National Cancer Institute (Bethesda, MD) for supplying JM8 for this study.
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suffering from stage III disease were treated at presentation with JM8. The median age of the patients was 58 years (range, 38-77).

Dose and Schedule

Patients were treated with a dose of 300 mg/m^2 of JM8, repeated at monthly intervals to a maximum of nine courses. The dose was reduced by increments of 50 mg/m^2 to a minimum of 200 mg/m^2 if any of the following risk factors were present: (*a*) glomerular filtration rate of < 60 ml/minute, (*b*) severe myelosuppression with previous therapy, or (*c*) age > 70 years. These three risk factors were shown in a previous phase I study to be associated with severe thrombocytopenia (6), and in the same study, tumor responses were seen with doses of JM8 as low as 120 mg/m^2. JM8 was administered in 500 ml of 5% dextrose over 30 minutes. There was no pre- or post-hydration.

Investigations

Patients were examined clinically prior to each course of therapy; in particular, any signs (either motor or sensory) of a peripheral neuropathy were recorded. At the time of the examination, patients were carefully questioned about their hearing, and in particular, whether their hearing had changed. Full blood cell counts were carried out immediately prior to each course of therapy and 3 weeks later to coincide with the previously described platelet count nadir. Serum urea and electrolytes were measured monthly and the glomerular filtration rate (GFR) was assessed by 51 Cr EDTA clearance (7) prior to the first course of therapy and, in 22 of the 24 patients who received three or more courses of JM8, after the third course of treatment. Audiograms were performed on nine randomly selected patients prior to the first course of treatment, and again three courses later.

Criteria for Response

Patients who received at least two courses of therapy at a minimum dose of 200 mg/m^2, and those who showed definite evidence of disease progression after one course of therapy, were considered evaluable for response. A complete response (CR) was defined as complete disappearance of all evidence of disease, clinically, ultrasonically, and radiographically, for at least 3 months. A partial response (PR) was defined as a 50% reduction in the size of all measurable lesions, including the disappearance of ascites, if present, without the appearance of new lesions. A minimal response was classified as "no response." Three patients, known to have residual disease after surgery, could not be assessed clinically and were therefore assessed for response by second-look operations.

RESULTS

Tumor Response

Patients Suffering from Recurrent Ovarian Carcinoma

As can be seen from table 1, 28 of the 33 pretreated patients suffering from recurrent ovarian carcinoma were assessable for tumor response. Two patients achieved a CR (duration, 6 and 5+ months) and five achieved a PR (duration, 7, 6, 5, 4, and 3 months).

Five patients were considered unevaluable for response. Four of these patients, all of whom had received previous myelosuppressive therapy, were unable to tolerate monthly treatment with JM8 at a dose of 200 mg/m^2 because of prolonged neutropenia or thrombocytopenia following their first course of treatment. A fifth patient died 3 weeks after her first course of therapy and was similarly considered unevaluable. It can be seen from table 2 that seven patients responded to JM8. Four of these seven responders were previously resistant to cisplatin, and a fifth patient had initially responded to, but subsequently relapsed, while receiving cisplatin. It is particularly interesting that tumor progression had occurred in three of these patients while receiving cisplatin (100 mg/m^2 of cisplatin, two patients; 50 mg/m^2 of cisplatin + 600 mg/m^2 of cyclophosphamide, one patient). Nevertheless, a definite response was seen in each case following JM8 given 1 month after the last dose of cisplatin.

Previously Untreated Stage III Patients

Again from table 1, it can be seen that all three stage III patients were clinically evaluable. One patient achieved a CR (duration, 3+ months) and one achieved a PR (duration, 4 months). The partial responder stopped treatment after five courses of therapy because coming into the hospital upset her life-style, and unfortunately, the disease relapsed 2 months later.

Toxicity

Bone Marrow Toxicity

It can be seen from table 3 that the peripheral wbc count fell to < 2000 × 10^9/L in six patients on one or

TABLE 1.—Response of patients with advanced ovarian carcinoma to JM8

Response	No. of patients	
	Recurrent stage*	Stage III†
CR	2	1
PR	5	1
No response	21	1
Not assessable	5	0

*Total patients = 33.
†Total patients = 3.

TABLE 2.—Response of JM8-sensitive patients to previous cisplatin

Response to JM8	Response to cisplatin	Cisplatin dose (mg/m^2) × No. of courses	Regimen	Other prior chemotherapy
CR	No response*	50 × 6	Cyclophosphamide + cisplatin	Cyclophosphamide alone, hexamethylmelamine alone
CR	Not assessable†	100 × 4	Cisplatin alone	Melphalan alone, chlorambucil alone
PR	CR	20 × 12*	Cisplatin + chlorambucil	—
PR	CR	20 × 12	Cisplatin + chlorambucil	Melphalan alone
PR	No response*	100 × 3	Cisplatin alone	—
PR	No response	120 × 4	Cisplatin alone	—
PR	No response*	100 × 5	Cisplatin alone	Cisplatin + chlorambucil

* Progressive disease while receiving cisplatin.
†No measurable disease at the end of surgery.

more occasions, and the platelet count fell to < 50,000 × 10^9/L in eight patients, again on one or more occasions. All of these patients had been heavily treated prior to JM8 therapy and, in fact, had received an average of 14 courses of three different chemotherapy regimens. In addition, of the 36 patients, 14 required blood transfusions during JM8 therapy. However, there was no myelosuppression in the three previously untreated patients.

Peripheral Neuropathy

Two patients, already experiencing a mild sensory peripheral neuropathy following cisplatin therapy, showed definite subjective and objective progression of this neuropathy while receiving JM8. Eight other patients, who were suffering from a mild platinum-induced peripheral neuropathy when JM8 was started, did not show any progression of the neuropathy while receiving this therapy. No patient developed a peripheral neuropathy for the first time while receiving JM8.

Hearing Loss

None of the 36 patients treated showed any subjective hearing loss with JM8 treatment, and none of the nine patients randomly selected for serial audiograms showed any evidence of deterioration in hearing while receiving treatment. However, probably as a result of cisplatin therapy, only two of these patients had normal audiograms at the start of JM8 therapy.

Renal Function

Twenty-two of the 24 patients who received three or more courses of JM8 had their 51 Cr EDTA clearances repeated after the third course of treatment. In 19 cases, the EDTA clearance either did not fall at all (16 patients), or fell < 20% of the original (three patients). In three patients, however, the EDTA clearance fell by > 20% of the original. In one of these patients, a subsequent EDTA clearance showed partial recovery in the GFR, and in one patient, progressive ovarian disease was demonstrated. There was no fall in the GFR in patients given only JM8.

Emesis

In this study, no attempt was made to record accurately the degree of vomiting. However, vomiting very rarely prevented discharge from the hospital on the day after treatment, and our general impression is that JM8 was better-tolerated than cisplatin.

DISCUSSION

A response rate of 25% in very heavily pretreated patients (seven of 28), all of whom had received cisplatin, and in two of three previously untreated patients suggests that JM8 is an active agent in the treatment of advanced ovarian carcinoma. Furthermore, a previous phase I study (6) has shown that previously untreated patients with good renal function can tolerate doses of JM8 well in excess of the doses used in this study, and thus, it may be that increasing the dose of JM8 may further enhance its antitumor efficacy.

It is particularly interesting that four of the seven patients who responded to JM8 had not responded to cisplatin, and that a fifth patient had initially responded to, but subsequently relapsed during, treatment with cispla-

TABLE 3.—Wbc and platelet count nadir with JM8

Wbc and platelet count nadir	No. of patients
Wbcs × 10^9/L	
> 3000	17
2000-3000	13
1000-2000	6
< 1000	0
Platelets × 10^9/L	
> 100,000	16
50,000-100,000	12
20,000-50,000	7
< 20,000	1

tin. This suggests that JM8 may sometimes be effective in cisplatin-resistant tumors (table 2).

The nonhematologic toxicity seen in this study was encouragingly low, with less neurotoxicity, ototoxicity, and renal toxicity than might have been expected from an analog of cisplatin. In particular, it was encouraging that only two of the ten patients suffering from cisplatin-induced peripheral neuropathy showed progression with JM8, and no patient showed any evidence of ototoxic effects. The degree of hematologic toxic effects was severe in some patients, but was acceptable, considering the multiple bone marrow-suppressing agents already received. At this late stage, most drugs, including cyclophosphamide, melphalan, etoposide, and hexamethylmelamine would produce similar severe falls in the wbc and platelet counts. However, the full blood cell count should be carefully monitored in all patients receiving JM8.

We conclude that JM8 is an active agent in the treatment of advanced ovarian carcinoma and may sometimes be effective against tumors which are resistant to cisplatin. Furthermore, these preliminary data suggest that JM8 may be less toxic than cisplatin. A randomized new clinical trial is therefore required to compare the antitumor efficacy and the relative toxic effects of these drugs in order to decide which of these platinum analogs should be the drug of choice in the treatment of ovarian carcinoma. As a result of this study, such a trial has been started at The Royal Marsden Hospital.

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Randomized Phase II Studies in Advanced Colorectal Carcinoma: A North Central Cancer Treatment Group Study[1,2]

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5-FU, semustine (MeCCNU), triazinate (TZT), and razoxane (ICRF-159) have each shown activity against advanced colorectal cancer in studies by at least two investigative groups. Objective response rates, however, have been low, without evidence of increased patient survival. The hope of this study was that enhanced activity might result from giving these agents in two-drug combinations. There were 167 eligible and evaluable patients randomized among the programs: 5-FU at a dose of 500 mg/m²/day by iv push × 5 (F); 5-FU at a dose of 400 mg/m²/day iv × 5 plus TZT at a dose of 175 mg/m²/day iv × 3 (FT); 5-FU at a dose of 400 mg/m²/day plus ICRF-159 at a dose of 600 mg/m²/day orally × 3 (FI); MeCCNU at a dose of 150 mg/m²/day orally plus TZT at a dose of 200 mg/m²/day iv × 3 (MT); MeCCNU at a dose of 150 mg/m² orally plus ICRF-159 at a dose of 500 mg/m²/day orally × 3 (MI); and ICRF-159 at a dose of 425 mg/m²/day orally × 3 plus TZT at a dose of 125 mg/m²/day iv × 3 (IT). Patients with limiting conditions (serum creatinine > 1.5 mg/dl or elevated bilirubin) were randomized among programs F, FI, and MI. Objective response rates by treatment arm were: F—13% (four of 31 patients); FT—13% (four of 31); FI—15% (four of 27); MT—11% (three of 28); MI—13% (four of 32); and IT—6% (one of 17). Response rates of combination arms were not significantly larger than those of 5-FU alone. With regard to survival, patients initially treated with 5-FU alone had the most favorable experience (median, 10.8 mos). Multivariate analysis showed the following factors to have a significant and independent influence on survival: Eastern Cooperative Oncology Group performance score, grade, site of indicator lesion, and the presence of 5-FU in the treatment regimen. Toxic effects most frequently seen were nausea, vomiting, thrombocytopenia, leukopenia, diarrhea, stomatitis, alopecia, and dermatitis. The incidence and severity of toxicity were roughly comparable among the six treatment arms. [Cancer Treat Rep 67:1001–1008, 1983]

Colorectal cancer is one of the most common malignant diseases encountered in the United States. Although a substantial proportion of patients with this disease will be cured by surgical treatment, most will either present with or later develop advanced and metastatic disease. Treatment of such patients is one of the most frequent challenges encountered in oncologic practice and thus far one of the least rewarding in terms of substantive benefit to the patient.

5-FU, with its very limited success, has been used for

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20 years in the treatment of advanced colorectal carcinoma. It has been extensively studied (by various routes and dose schedules) and has produced objective response rates generally in the range of 15%-20%. When responses occur they are usually not complete and are of relatively short duration. There has been no consistent evidence establishing that 5-FU therapy will improve patient survival regardless of the stage of disease at which it is applied.

The nitrosoureas have shown definite but limited activity in studies by a number of investigators treating colorectal cancer. Objective response rates have generally been in the 10%-15% range. In a nonrandomized Mayo Clinic experience, semustine (MeCCNU) has produced response rates slightly higher than lomustine or carmustine (1). In a randomized study, the Cancer and Acute Leukemia Group B found MeCCNU to produce more responses in colorectal cancer than lomustine.[14]

Razoxane (ICRF-159), an orally administered piperazine derivative, has also been found to have modest activity against colorectal cancer. Marciniak et al (2) observed three responses among 25 patients (12%) and Bellet et al (3) reported five responses among 35 patients (14.3%). This drug is well-tolerated orally, and leukopenia has been the only important toxic effect.

Triazinate (TZT) is a potent inhibitor of dihydrofolate reductase and is similar to, but distinct from, methotrexate. Phase II trials done at the M. D. Anderson Hospital and at the Mayo Clinic produced objective response rates of 16% (four of 25 patients) and 18% (five of 28), respectively, in patients with advanced colorectal cancer (4,5).

Due to the prevalence of advanced colorectal cancer, the dearth of effective treatment for this disease, and the modest activity of the single agents, a phase II trial of possible two-drug combinations of these agents was considered appropriate. This study was conducted in a community setting by the North Central Cancer Treatment Group.

Before initiating this randomized trial, pilot studies were conducted at the Mayo Clinic to develop dose regimens for the combinations which produced definite but clinically tolerable toxicity.

MATERIALS AND METHODS

Patient Selection

Entry of patients was restricted to those with histologically proven unresectable or metastatic colorectal cancer without prior chemotherapy. The patients were required to have a measurable area of known malignant disease to serve as an objective indicator of response to therapy. A measurable tumor was defined as a mass which could be measured on physical examination or on chest x-ray with a ruler or calipers. Rectal or pelvic masses, lesions found on barium studies or ultrasonic scans, and ascites were not considered measurable lesions. Malignant hepatomegaly was acceptable as a measurable lesion if the liver edge extended at least 5 cm below the xiphoid process or a costal margin on quiet respiration. A positive liver scan in the absence of hepatomegaly on the physical examination was permitted if there was a clearly defined perfusion defect measuring at least 5 cm in greatest diameter. Patients were required to be ambulatory with a reasonable state of nutrition. A wbc count of $> 4000/mm^3$ and a platelet count of $> 100,000/mm^3$ were required. Patients had an Eastern Cooperative Oncology Group (ECOG) score ≤ 3 and were maintaining a daily intake of at least 1200 calories. Significant infection, frequent vomiting, or inadequate veins excluded patients from the study. Convulsive disorders, active cerebrovascular disease, serum creatinine of > 1.5 mg/dl, or any elevation of direct-reacting bilirubin were limiting conditions, which did not allow patients to be randomized to a TZT-containing regimen. Entry into the study was deferred until 4 weeks after major surgery or radiation therapy. Informed consent for investigational treatment was obtained prior to patient entry.

Randomization Procedure

An adaptive randomization procedure was used for each participating institution. Patients were stratified according to ECOG performance status (0-1 or 2-3) and site of primary indicator lesion (hepatic, pulmonary, other). Patients with limiting conditions were randomized among 5-FU (F), 5-FU plus ICRF-159 (FI), and MeCCNU plus ICRF-159 (MI). Patients without limiting conditions were randomized among six regimens, consisting of the combinations listed above as well as 5-FU plus TZT (FT), MeCCNU plus TZT (MT), and ICRF-159 plus TZT (IT).

Study Endpoints

Response rates, survival time, and time to progression were analyzed.

Objective response categories included complete response, partial response, stable disease, and disease progression. Complete response was defined as the absence of any clinically detectable tumor. Partial response was defined as a reduction by at least 50% of the product of the longest perpendicular diameters of the primary indicator lesion. If hepatomegaly was the indicator lesion there must have been at least a 30% reduction of the sum of liver measurements below the costal margins at the midclavicular line and xiphoid process. There could be no increase in any other indicator lesion, nor could there be new areas of malignant disease. Disease progression was defined as a $> 25\%$ increase from the best response in the

[14]Martz G, Maurice P, and Holland JF, for Cancer and Acute Leukemia Group B—1975. Personal communication.

product of longest perpendicular diameters of the primary indicator lesion or the appearance of a new area of malignant disease. Stable disease was defined as any other outcome. Response rate was defined as the percentage of complete and partial responses in the number of patients evaluable for response.

Survival was measured from the first day of treatment to death or last follow-up.

Progression time was measured from the first day of treatment to the day of documented tumor progression. If no progression was noted, then the progression time is censored at the date the patient was taken off the study.

Treatment Methodology

Prior to initial and subsequent treatment the following examinations and tests were prescribed: history and physical examination, wbc count, platelet count, hemoglobin, chemistry group (including SGOT, serum creatinine, alkaline phosphatase, and direct and total bilirubin), chest x-ray, and urinalysis.

Dosages and routes for each of the six regimens are given in table 1. Dosages for later courses were reduced systematically if excessive toxicity was observed in the preceding course. Wbc and platelet counts were taken on the final day of each treatment course, twice weekly during the first cycle, and once weekly during subsequent cycles.

If none of the first 15 patients on the IT, MT, or MI arms achieved a complete or partial response, then that treatment arm was to be dropped.

Treatment was continued until disease progression. Upon progression on arms F, FT, FI, MI, MT, or IT, patients, if eligible, were directly assigned to arms IT, MI, MT, FT, FI, and F, respectively.

Statistical Methods

A Kruskal-Wallis test was used to compare age distributions among treatment regimens for patients with no limiting condition (6). Chi-square tests for homogeneity were performed to compare distributions of the covariates sex, grade, primary indicator lesion, and performance score among the treatment arms. Survival and progression time distributions were estimated by the Kaplan-Meier method (7). Differences among survival and progression distributions were tested by log-rank tests (8). Cox covariate analyses were performed to assess joint influence of the covariates using the patients entering without limiting conditions (9). The covariates evaluated by using the Cox model in addition to stratification factors were age (< 63 vs ≥ 63 years), sex, grade (1, 2 vs 3, 4), treatment assigned, and the two-factor interactions of all previous variables. Due to small numbers of patients entering with limiting conditions, a Cox analysis was not performed.

Logistic regression models (10) were used to assess the influence of the covariates given above on the binary variable objective response (complete responses plus partial responses vs no response). The logistic regression was performed only on the no-limiting-condition subset.

RESULTS

Patient Evaluability

One hundred and eighty-seven patients were randomized. Eighteen patients were declared ineligible (15 due to lack of biopsy-proven recurrent or metastatic disease, one due to radiation therapy given within 4 weeks prior

TABLE 1.—Treatment schedules

Arm	Agent	Dose (mg/m^2/day)	Days	Frequency
F	5-FU	500	1-5	q 5 wks
FT	5-FU	400	1-5	q 5 wks
	+			
	TZT	175	1-3	
FI	5-FU	400	1-5	q 5 wks
	+			
	ICRF-159	600	1-3	
MI	MeCCNU	150	1	q 8 wks
	+			
	ICRF-159	500	2-4 29-31	
MT	MeCCNU	150	1	q 8 wks
	+			
	TZT	200	1-3 29-31	
IT	ICRF-159	425	1-3	q 4 wks
	+			
	TZT	125	1-3	

TABLE 2.—Pretreatment characteristics of patients with and without limiting conditions*

	Treatment arm					
	F	FT	FI	MI	MT	IT
Total patients	31	31	28	30	30	17
Age (yrs) at randomization						
Median	64.0	64.0	58.5	64.5	63.5	63.0
Range	36-75	36-83	40-77	48-78	51-74	36-83
Sex						
Male	22	15	12	20	17	8
Female	9	16	16	10	13	9
Performance score						
0 or 1	24	23	21	21	20	13
2 or 3	7	8	7	9	10	4
Primary indicator lesion						
Hepatic	17	17	16	16	18	9
Pulmonary	10	10	8	8	8	5
Other	4	4	4	6	4	3
Grade						
1 or 2	23	21	19	22	22	15
3 or 4	8	10	9	8	8	2

* Unless specified otherwise, values = No. of patients.

to treatment, one because the patient had no measurable disease, and one because metastatic disease was identified to have originated from a kidney primary). One patient refused to be treated after randomization and thus was dropped from study. One patient was disqualified because of a major violation in treatment administration. These 20 patients were excluded from further analysis. With all exclusions, 167 patients were eligible. One patient randomized to FI refused to return for evaluation after the first treatment. This patient was not evaluable for response or progression but was evaluable for survival. The 166 other patients were evaluable for response, survival, and progression. Six patients entered with limiting conditions.

Fifty-six patients were able to receive cross-over treatment.

Patient Characteristics

Pretreatment characteristics of the 167 eligible and evaluable patients are presented in table 2. ECOG performance score and primary indicator lesion were stratification factors and were well-balanced among the six treatment arms. There was some imbalance in sex distribution, but age as well as grade were remarkably well-balanced.

Response

Of the 160 patients without limiting conditions who were evaluable for response, 20 (12.5%) responded. Only 17 patients were entered on treatment arm IT. There were no responses among the first 15 evaluable patients and, as per protocol, the arm was closed to entry. However, at the time the arm was closed, two additional patients had not been completely observed for therapeutic response. One of these last two patients responded.

Ninety patients entered with hepatic metastases as the primary indicator lesion. Thirty-seven patients were evaluated for response using only serial liver size as measured on serial physical examinations. Fifty-three patients had response assessment via serial liver scans. Of these 53 patients, 83% had scans every 5 weeks until disease progression, while the other 17% had scans approximately every 10 weeks.

Observed regression rates of the combination arms (table 3) were not significantly different from the 5-FU-alone arm (chi-square P values > 0.40). No responses were found among the six patients entering with limiting conditions.

Covariates influencing response were site of primary indicator lesion and sex. Being female or having a nonpulmonary lesion as the indicator was indicative of being

TABLE 3.—Regression rates of patients without limiting conditions

Covariate	Regression rate*
Treatment	
5-FU	4/29 (13.7)
5-FU + TZT	4/31 (12.9)
5-FU + ICRF-159	4/25 (16.0)
MeCCNU + ICRF-159	3/28 (10.7)
MeCCNU + TZT	4/30 (13.3)
TZT + ICRF-159	1/17 (5.8)
Sex	
Male	6/89 (6.7)
Female	14/71 (19.7)
Primary indicator lesion	
Hepatic	13/90 (14.4)
Pulmonary	1/48 (2.1)
Other	6/22 (27.3)

* No. of patients with regression/No. treated (%).

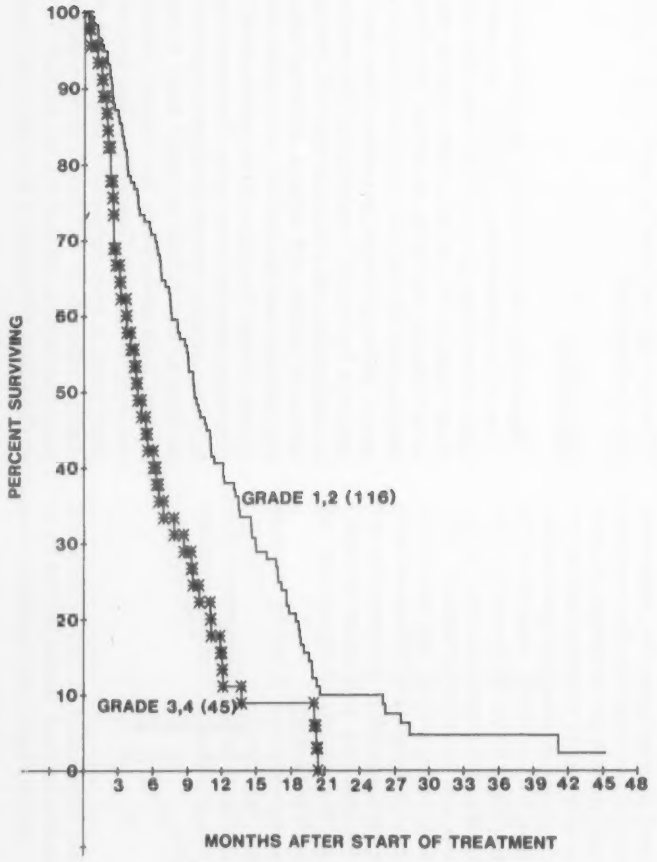

FIGURE 1.—Survival among patients without limiting conditions, by grade.

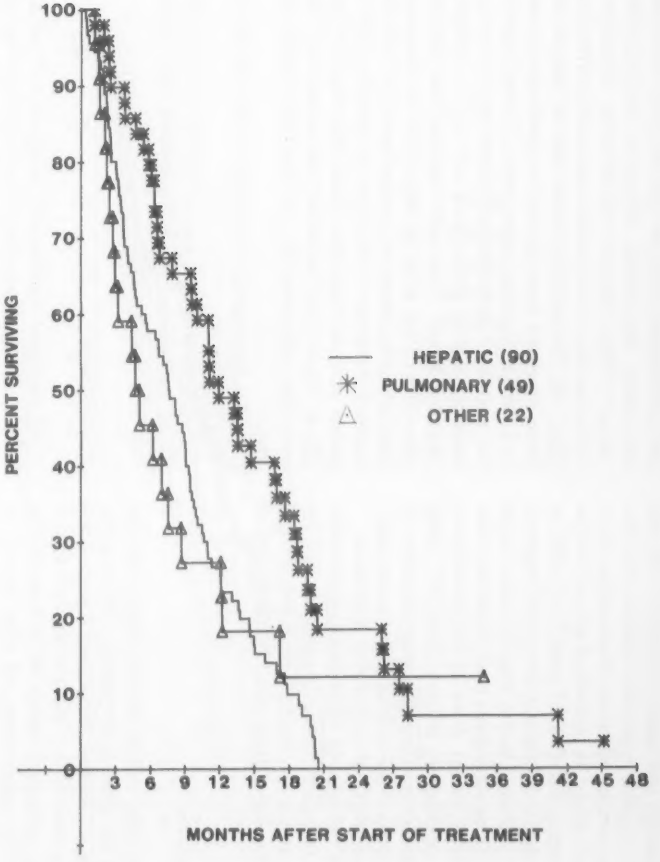

FIGURE 2.—Survival among patients without limiting conditions, by primary indicator lesion.

more likely to respond (chi-square P values = 0.01 and 0.01; table 3). A logistic regression analysis was performed to assess the joint influence of covariates on response. In the joint model, sex and site of primary indicator lesion were significant (P = 0.01 and P = 0.003, respectively). The chance of response given good prognosis (female, "other" indicator lesion) was 44 times larger than the chance of response given poor prognosis (male, pulmonary indicator lesion). None of the combination treatments was significantly different from 5-FU alone even after adjustment for sex and primary indicator lesion (P = 0.80). None of the interaction terms was significant after adjustment for the significant covariates.

Response rates for patients able to receive cross-over treatment were 14% for F (two of 14 patients); 11% for FT (one of nine); 6% for FI (one of 17); 0% for MI (none of five); 0% for MT (none of five); and 17% for IT (one of six).

Disease Progression

Of the 160 patients without limiting conditions who were analyzable for time to progression, 142 had progressed at the time of analysis. Patients with grade 3 or 4 and performance score of 2-3 had shorter time to progression. Having pulmonary lesions led to delayed time to progression over hepatic sites and other sites (P = 0.03 in each model). After adjustment for grade and performance score, site of indicator lesion failed to be significant (adjusted, P = 0.07). The progression hazard rate of patients with poor prognosis (grade 3-4, performance score of 2-3) was 2.9 times larger than the progression hazard rate for patients with the best prognosis (grade 1-2, performance score of 0-1). Treatment assigned was significant alone and after adjustment for grade and performance score (P = 0.02 and 0.004, respectively). Patients with treatments MT, MI, and IT (the non-5-FU-containing regimens) had earlier progression times. The only interaction of note was grade by performance score. Those patients with high grade and high performance score did worse than all of the others. Differences among the other three levels determined by the interaction were not significant. We must view with caution the significance of these results since only 13 patients had high grade and high performance score.

Median time to progression was 38 days for the six patients with limiting conditions.

Only performance score influenced progression times among the patients who received cross-over treatment (log-rank, P = 0.02).

Survival

One hundred and forty-nine of the 161 patients without limiting conditions are dead. Median survival for all patients was 8.5 months. Grade, site of indicator lesion, and performance score are the covariates which alone and jointly significantly influenced survival (joint P values < 0.003). Patients with low grade, pulmonary indicator lesions, or low performance score survived longer (figs 1-3). The mortality hazard rate for patients in the worst prognosis group (grade 3-4, performance score of 2-3, "other" indicator lesion) was 4.7 times larger than the mortality hazard rate for patients with the best prognosis (grade 1-2, performance score of 0-1, pulmonary indicator lesion). Treatment assigned was significant alone (P = 0.014). After adjustment for the three significant covariates, treatment is significant (P = 0.05). Here, patients receiving combinations not including 5-FU had poorer survival times than those receiving 5-FU alone (fig 4). None of the two-factor interactions was significant after adjustment for the significant single covariates.

Median survival was 3.8 months for patients with limiting conditions.

Median survival time was 4.3 months for cross-over patients, and survival was not associated with treatment (log-rank P value = 0.21).

Toxic Effects

All treatment combinations produced leukopenia and thrombocytopenia but rarely of a life-threatening degree (table 4). Nonhematologic toxic effects consisted of nausea, vomiting, diarrhea, and stomatitis. These too were generally not severe or life-threatening (table 5). The 5-FU-containing arms tended to produce more frequent diarrhea and stomatitis.

DISCUSSION

Regrettably, none of the results obtained from the use of any of the combinations in this study are superior to those obtained from the use of 5-FU alone. The addition of any drugs to 5-FU did not improve response rate, interval to disease progression, or survival. It is of interest that treatment with the 5-FU regimens resulted in longer survival times and delayed progression times than treatment with the non-5-FU combinations. This result could be interpreted as indicating either that 5-FU does add to survival of patients with advanced colorectal cancer or that treatment with the non-5-FU combinations detracted from survival. Resolution of this question would have required an untreated control group. Randomized studies involving untreated controls for advanced colorectal cancer would be very difficult to conduct today. In surgical adjuvant trials conducted by the Veterans Administration Surgical Adjuvant Group (11), which included patients found to have unresectable disease, there was a survival advantage of 5-FU- or floxuridine-treated patients over controls, although the case numbers were too small for these differences to be accorded statistical sig-

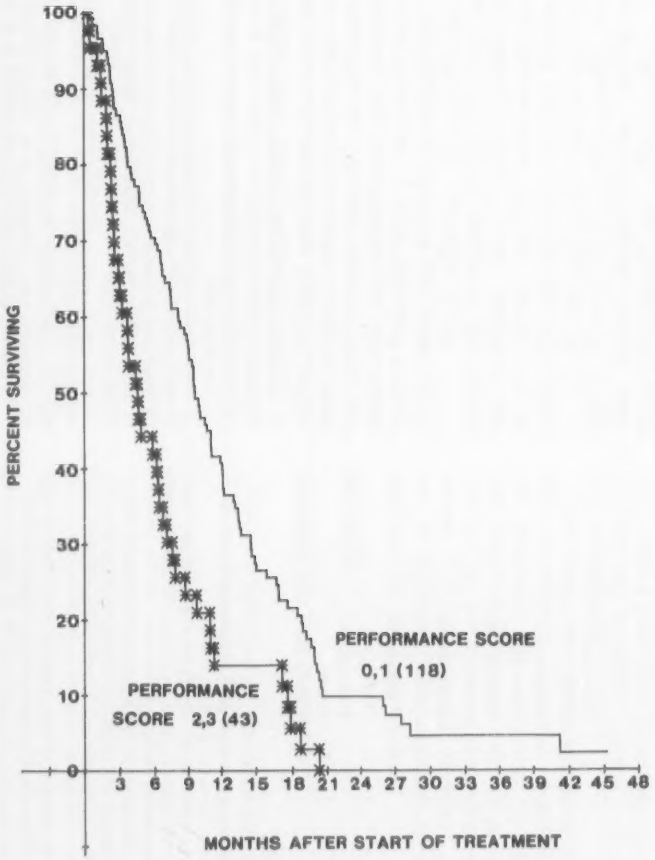

FIGURE 3.—Survival among patients without limiting conditions, by performance score.

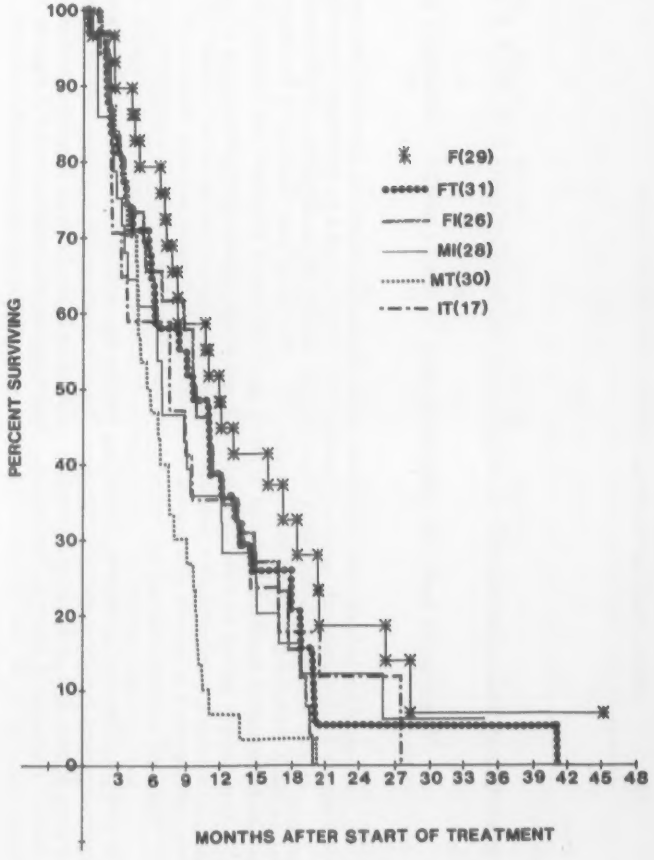

FIGURE 4.—Survival among patients without limiting conditions, by treatment.

TABLE 4.—Hematologic toxic effects of patients without limiting conditions.

Toxic effect	% of patients from treatment arm— F	FT	FI	MI	MT	IT
Leukopenia (cells/mm^3)						
1st cycle	(*N* = 29)	(*N* = 29)	(*N* = 31)	(*N* = 22)	(*N* = 26)	(*N* = 17)
Wbc count nadir < 4000 but ⩾ 1500	76	58	59	54	57	41
Wbc count nadir < 1500	17	10	32	14	0	0
All cycles	(*N* = 29)	(*N* = 31)	(*N* = 23)	(*N* = 26)	(*N* = 30)	(*N* = 17)
Wbc count nadir < 4000 but ⩾ 1500	79	84	61	77	60	71
Wbc count nadir < 1500	21	10	30	15	3	0
Thrombocytopenia (cells/mm^3)						
1st cycle	(*N* = 29)	(*N* = 31)	(*N* = 22)	(*N* = 25)	(*N* = 28)	(*N* = 17)
Platelet count nadir < 130,000 but ⩾ 50,000	14	32	36	48	40	6
Platelet count nadir < 50,000	0	3	5	8	13	0
All cycles	(*N* = 29)	(*N* = 31)	(*N* = 23)	(*N* = 26)	(*N* = 30)	(*N* = 17)
Platelet count nadir < 130,000 but ⩾ 50,000	66	55	44	54	47	12
Platelet count nadir < 50,000	0	3	4	12	20	0

nificance. The striking differences in response rates of pulmonary lesions compared to other measurable sites of tumor are of some interest and could either represent preferential biologic resistance of tumor in the lung to our chemotherapy regimens or the fact that pulmonary nodules can usually be measured with more precision. The only firm conclusion we can draw from our data is that there is no advantage to using any of the two-drug combinations we studied over 5-FU alone in the treatment of patients with advanced colorectal cancer.

TABLE 5.—Nonhematologic toxic effects

Toxic effect	Degree	% of patients from treatment arm— F (*N* = 29)	FT (*N* = 31)	FI (*N* = 26)	MI (*N* = 28)	MT (*N* = 30)	IT (*N* = 17)
Nausea	Mild/moderate	52	54	35	54	60	65
	Severe	7	3	0	0	7	0
Vomiting	Mild/moderate	25	42	37	46	46	30
	Severe	4	3	0	0	7	0
Diarrhea	Mild/moderate	52	23	16	4	23	12
	Severe	10	13	0	0	3	0
Stomatitis	Mild/moderate	51	42	27	4	10	0
	Severe	21	23	0	0	7	0
Dermatitis	Mild/moderate	10	23	0	4	3	6
	Severe	0	13	0	0	7	0
Alopecia	Mild/moderate	21	13	8	11	16	0
	Severe	3	6	19	0	0	12

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Phase II Trial of Lymphoblastoid Interferon in Metastatic Colon Carcinoma[1,2,3]

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Nineteen patients with advanced metastatic colon carcinoma were treated with human lymphoblastoid interferon at a dose of 3.0×10^6 units/m^2 im three times/week for 6 weeks. Patients who did not progress at the 6-week interval were randomized to receive maintenance treatment with either interferon (3.0×10^6 units/m^2 once/week) or no further treatment. All patients had evaluable metastatic lung, liver, or abdominal disease as measured by radiographs or computerized tomographic scans. Complete remission or partial remission of > 50% decrease in tumor measurements was not demonstrated with interferon treatment. Among 18 evaluable patients, seven had stabilization of measurable disease at 6 weeks, but 11 showed progressive disease. Of the seven patients followed during maintenance, only one (placebo maintenance) remained with no objective progression. No serious organ toxic effects were attributed to interferon, but one patient demonstrated liver enzyme elevation that persisted after cessation of interferon therapy. All patients had significant constitutional symptoms of fever, muscle aches, and malaise. Using interferon at the dose schedule outlined, this study failed to demonstrate significant regressions in metastatic colon carcinoma. [Cancer Treat Rep 67:1009–1012, 1983]

Interferon, a naturally occurring protein, has potent antiviral, antiproliferative, and immunomodulating properties (1). The recent availability of alpha, beta, and gamma interferons and various sources of each variety has made uniform studies with this biologic response modifier difficult. Clinical studies with leukocyte-derived interferon in neoplastic diseases such as osteogenic sarcoma, breast cancer, myeloma, and lymphoma have been limited because of the scarcity and impurity of this source of interferon (2,3).

Human lymphoblastoid interferon (Wellferon), a more homogeneous preparation of alpha interferon purified from a human B cell lymphoblastoid line, is produced in large quantities and has been studied in early clinical and in vitro studies (4). A recently completed phase I study has shown the limiting toxic effects of this immunomodulator when given in escalating doses (5). In the course of that study, two patients with progressive metastatic colon carcinoma demonstrated clinical benefit from the 5 weeks of interferon administration. Another phase I study with recombinant interferon demonstrated only one partial response in 20 patients with colon carcinoma (6).

Conventional chemotherapeutic agents have produced objective remissions in a small proportion of patients with metastatic colon carcinoma, but the responses have been of short duration with little influence on survival (7). For this reason, a therapeutic approach was assessed with lymphoblastoid interferon in the treatment of metastatic colon carcinoma. In addition, the toxicity of this agent was evaluated.

METHODS

Interferon and Trial Design

Human lymphoblastoid interferon (produced by the Wellcome Foundation, Ltd, Beckenham, Kent, England)

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was prepared from cells of the human B cell lymphoblastoid line, Namalva, stimulated with Sendai virus. The preparation was purified to a level of 2×10^8 international units of human alpha interferon per milligram of protein and was supplied frozen in tris-glycine-buffered physiologic saline. Interferon was stored at -20°C, then thawed and stored at 2-8°C for no longer than 2 weeks before use.

The trial consisted of an initial 42-day period of im interferon (3.0×10^6 units/m^2) three times/week (Monday, Wednesday, and Friday). Progressive disease demonstrated at 28 or 42 days of therapy terminated the trial for any patient. The criterion for a 50% dose reduction for constitutional symptoms was based on 2 weeks of incapacitating weakness requiring bed confinement. Patients whose disease did not progress during treatment were randomized to receive either weekly maintenance interferon (3.0×10^6 units/m^2) or to discontinue interferon. The response was then evaluated at 4-week intervals until disease progression.

Patient Selection and Evaluation

Eligible patients had a histologically documented diagnosis of recurrent or metastatic adenocarcinoma of the colon or rectum, with good performance status (Karnofsky status > 60%), and no history of other malignant neoplasms. Patients had either no prior chemotherapy or therapy no less than 4 weeks prior to beginning interferon. All patients had measurable disease, including evaluable superficial metastatic nodules or lung, liver, or abdominal disease measurable by radiographs or scans. Computed tomograms were performed at 1-cm continuous intervals through the region of the tumor. Patients with a hemoglobin count < 8 g/100 ml, absolute granulocyte count < 750/μl, platelet count < 50,000/μl, bilirubin > 2.5 mg/100 ml, SGOT > 100 units, albumin < 2.5 g/100 ml, or prothrombin time > 1.5 times control were excluded from the study.

Nineteen patients gave written informed consent to participate in the study. All patients were evaluated every 2 weeks by the investigators at Duke University; some patients received their interferon injections (Monday, Wednesday, and Friday) from the personal physician. All of the doses for injections were formulated at this institution by a pharmacist.

Definitions of response in metastatic colon carcinoma relied on reproducible measurements of the sum of the products of perpendicular diameters of all nodular and radiographic lesions. A partial response (PR) was defined as a ≥ 50% reduction compared with the original measurements. Disease progression was defined as a > 25% increase in measured lesions or development of new lesions. Stabilization was defined as disease which fell between the criteria for PR and progression. Symptomatic toxicity was quantitated according to Southeastern Cooperative Cancer Study Group criteria. Patients were closely monitored for hepatic, hematologic, and renal toxic effects. Serial CEA were also determined.

RESULTS

There were 11 male and eight female patients, with a mean age of 58 years (range, 26-84). The mean Karnofsky performance status was 85%. All patients had distant metastases from a histologically demonstrated colon/rectal carcinoma, and all had evaluable subcutaneous, lung, liver, or abdominal disease. One patient was inevaluable after treatment for 1 week because he died from new pleural effusion and presumed sepsis. Seven patients had prior treatment other than earlier surgery to stage their disease. Six patients had received chemotherapy, and two had received radiation therapy. All patients received interferon injections three times weekly for the first 6 weeks or until objective progression.

Of the 18 evaluable patients, no complete or partial responses were attained. Eleven of the 18 patients had progressive disease either before or at the 6-week point. At the 6-week evaluation interval, seven patients had objective stabilization (tumor neither regressed nor progressed > 25% from the original measurement). These patients were randomized to either weekly interferon or no further therapy. Most patients followed during this maintenance period progressed within 10-14 weeks after beginning the interferon study. One patient, however, continues to be followed with no progression 32+ weeks after therapy. This patient had been randomized to receive no interferon maintenance therapy.

Measurable disease was assessed by serial computed tomogram of the abdomen (16 patients), chest radiographs (ten), or both (nine). Before interferon therapy, elevated CEA were found in 16 patients. Nine patients in whom the CEA increased by > 25% during the 6-10-week interval had objective progression. Four patients who were clinically stable 10 weeks after treatment had a decrease or stability in CEA levels. However, the CEA level decreased in three cases when metastatis had progressed.

Toxicity

All patients were evaluated for toxic effects. The major toxic effects encountered during 6 weeks of interferon administration were the constitutional effects of fever, chills, fatigue, anorexia, malaise, myalgia, and weight loss (table 1). No patient lost > 10% of original body weight. Four patients experienced fever, chills, lethargy, and incapacitating weakness for > 2 weeks that required a 50% dose reduction. Improvement in

TABLE 1.—Symptomatic toxicity of interferon experienced between 2 and 6 wks of treatment

Toxic effect	No. of patients*
Gastrointestinal (mild nausea/± vomiting)	10
Weight loss†	18
Fever	17
Anorexia	15
Fatigue	17
Malaise	15
Myalgia	13
Headache	14
Arthralgia	9

*Total = 18.
†No patient lost > 10% of original body weight during treatment.

these symptoms followed, and the four patients were able to continue at this reduced dose. Temperature elevation occurred in all patients on the first day of therapy, and continued episodically during the next 6 weeks in 17 of 18 patients. No organ or chemical toxic effects were sufficiently severe during treatment to require cessation of interferon therapy.

Moderate leukopenia was common during interferon therapy (table 2). A pretherapy leukocyte count of > 3200/mm^3 occurred in all 18 patients. Granulocyte counts (mean decrease, 1452/mm^3) were more depressed than lymphocyte counts (mean decrease, 158/mm^3). A significant depression in the mean hemoglobin did not occur. The largest decrease in hemoglobin in any one patient was 1.8 g/dl. No patient required rbc transfusion. Most patients showed a small decrease in platelet counts. Bone marrow depression was not dose-limiting in any patients, and the mild granulocyte and platelet suppression was reversible after cessation of the interferon.

Thirteen patients had liver metastasis as major measurable disease, with nine patients having pretreatment elevation in alkaline phosphatase and transaminases. A significant increase in liver enzymes after the course of interferon treatment was not demonstrated in patients with normal pretreatment levels. All patients with further increase in alkaline phosphatase or transaminases after interferon therapy had evidence of progressive hepatic disease. One patient classified as having stable radiographic hepatic disease demonstrated a persistent elevation of alkaline phosphatase and LDH after interferon was stopped. This patient was found to have progression of hepatic metastases 11 weeks later. Hepatic or renal toxic effects were not dose-limiting in any patients.

DISCUSSION

The data from this phase II study in metastatic colon carcinoma suggest that human lymphoblastoid interferon at the dose schedule outlined is not effective in inducing regression of established metastasis. Indeed, the lack of tumor regression caused premature termination of this study when it was evident that there was a < 5% chance of missing a program having 15% effectiveness by this dose schedule. This is disappointing in light of the recent reports of encouraging in vitro antiproliferative effects and clinical response in breast, myeloma, lymphoma, and renal malignancies (2,3,8), although not all of the reports are positive (6,9–11).

A recently completed phase I study has described the clinical, pharmacologic, and immune responses of patients with cancer to human lymphoblastoid interferon (5). That study included rapid dose escalation of interferon, and helped establish the dose and schedule of interferon that were used in the current study. At a dose of 3.0×10^6 units/m^2, it was thought that interferon represented a regimen that would have clinical practicality and acceptable toxicity, and still provide some natural killer cell stimulation. This immune response is thought to be one of the important mechanisms in the antineoplastic effect of interferon (12–15). For the clinical application of interferon, several variables must be considered: concentration in specific body fluids, dose, type of interferon, route, inter-

TABLE 2.—Toxic effects of lymphoblastoid interferon

	Pretherapy*	Posttherapy*,†
Leukocytes/mm^3	6844 (10,000–3200)	4961 (12,700–2900)
Granulocytes/mm^3	4593 (7392–1952)	3141 (9652–1000)
Lymphocytes/mm^3	1433 (3744–444)	1275 (2159–76)
Hemoglobin, g/dl	13.3 (16.0–10.5)	12.8 (14.5–9.2)
Platelets/mm^3	328 (567–197)	240 (793–108)

*Values in parentheses = range.
†Posttherapy values are lowest values recorded 2–6 wks after interferon therapy.

val of administration, and combination with other drugs.

Our study found constitutional symptoms with interferon that were similar to those with different doses and schedules (4,5). All patients initially developed fever, chills, and malaise, and these symptoms continued, although the fever and chills declined to a reduced level. In our study, only four of 18 patients had constitutional effects severe enough to warrant a dose reduction. A mild and reversible depression in granulocytes and platelets occurred, but did not warrant a dose reduction. Liver enzyme elevations were attributed to progressive hepatic disease from metastasis. However, one patient with stable liver disease demonstrated a prolonged increase in liver enzyme after interferon treatment was stopped.

We conclude that human lymphoblastoid interferon given in the above regimen is a tolerable program which causes constitutional and minimal organ toxic effects, but does not stop the progression of metastatic colon carcinoma. Further studies at higher doses of interferon are being performed in patients with colon cancer at other institutions.

ADDENDUM

Two other papers presented at the ASCO meeting showed lack of response of interferon in metastatic colon cancer: Neefe et al (Proc ASCO 2:52, 1983) had one brief PR and 17 progressions among 18 evaluable patients treated with recombinant clone A interferon; Figlin and Sarna (Proc ASCO 2:45, 1983) reported no responses among 18 evaluable patients treated with human leukocyte interferon.

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Lack of Dexamethasone Effect on the Antitumor Activity of Cisplatin[1,2]

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High-dose glucocorticosteroids have proven to be effective antiemetic agents when used before and after cisplatin chemotherapy. To rule out a possible decrease in cisplatin antitumor activity by a direct effect of short-term glucocorticosteroid treatment, survival was studied in mice inoculated with P388 leukemia and then treated with cisplatin preceded by a single high dose (1.5 mg/kg) of dexamethasone (Dex). The median survival of animals treated with cisplatin-Dex was 19 days, and that of animals treated with cisplatin alone was 18 days. These survival durations were significantly longer ($P < 0.01$) than the survival time of the controls (median, 12 days). A double-layer soft agar clonogenic assay was used to study the possible effect of Dex on cisplatin inhibition of tumor colony-forming units from two human tumor cell lines (HEC-1A, endometrial; and WiDr, colon). In this model, the addition of Dex to cisplatin showed no statistically significant effect. These data suggest that Dex has no inhibitory effect on the antitumor activity of cisplatin. [Cancer Treat Rep 67:1013–1017, 1983]

We (1) and others (2,3) have shown recently that high-dose oral dexamethasone (Dex) is an effective antiemetic in cancer patients receiving chemotherapy including cisplatin. There is, however, concern regarding the possible inhibition by glucocorticosteroids of cisplatin's cytotoxic effects (4). For example, the activity of cisplatin against Sarcoma 180 appears to be decreased by prolonged immunosuppression with hydrocortisone (5). The typical dosing schedule for Dex as an antiemetic involves only a short-term exposure which is unlikely to have serious immunosuppressive effects. Because of the possibility that Dex might inhibit cisplatin cytotoxicity, we have used an in vivo mouse tumor model and an in vitro human tumor clonogenic assay to study this potentially adverse interaction.

MATERIALS AND METHODS

In Vivo Studies

Male DBA/2 mice (Jackson Laboratories, Bar Harbor, ME), 10–12 weeks old, were injected ip with 10^6 P388 leukemia cells (obtained from Dr. Robert Struck, Southern Research Institute, Birmingham, AL). Twenty-four hours later, mice were injected with one of the following: saline (five mice); Dex, 1.5 mg/kg (ten); cisplatin, 5 mg/kg (ten); Dex followed by cisplatin 10 minutes later (ten); or a combination of cisplatin and Dex that had been mixed in vitro and kept at room temperature for 1 hour prior to administration (ten). The animals were observed twice daily for survival until all animals had died.

In Vitro Studies

Cell lines.—HEC-1A, a human endometrial cancer cell line (6), was originally obtained from Dr. Jorgen Fogh, Memorial Sloan-Kettering Cancer Center, NY, and was maintained in McCoy's 5A medium (Gibco Laboratories, Grand Island, NY) with 10% fetal calf serum (Gibco) and 1% penicillin (10,000 units/ml)-streptomycin (10,000 μg/ml (Gibco). WiDr (CCL 218), a human colon adenocarcinoma cell line (7), was obtained from Dr. Anne Hamburger (American Type Culture Collection, Rockville, MD) and was maintained in RPMI-1640 medium (Gibco) with

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10% fetal calf serum and 1% penicillin-streptomycin.

Soft agar tumor cloning methods.—A double-layer soft agar clonogenic assay similar to that described by Soehnlen et al was used (8). Briefly, the cell lines were harvested using either Tyrode's Solution (HEC-1A) (9) or 0.25 trypsin-EDTA (Gibco) (WiDr). After two washings in Mccoy's 5A medium, aliquots of the single-cell suspensions were exposed in culture medium to Dex (1 μg/ml), cisplatin (various concentrations between 0.1 and 10 μg/ml), or the two-drug combination for 1 hour; then washed three times; and plated in their respective media with 0.3 agar over an 0.5 agar-culture medium underlayer in 35-mm Petri dishes. For continuous exposure studies, the drugs were added to the cell suspension before plating at a Dex concentration of 0.5 μg/ml and cisplatin concentrations varying between 0.005 and 0.5 μg/ml. We also conducted experiments with a 24-hour pre-exposure of the tumor cells to Dex (0.5 μg/ml).

Colony counting.—Tumor colony-forming units (TCFU) (ie, colonies of ⩾ 60 μm) were counted using the FAS II Omnicon Bausch and Lomb automated image analyzer that had been optimized for tumor colony counting.

Calculations

The survival curves of leukemic mice were studied with both Breslow and log-rank tests. Results of the in vitro experiments were expressed as percent TCFU ± SE compared to the controls. Each individual concentration tested with or without Dex was compared according to a Z-test, as described by Drewinko et al (10), and the dose-response curves were analyzed similarly.

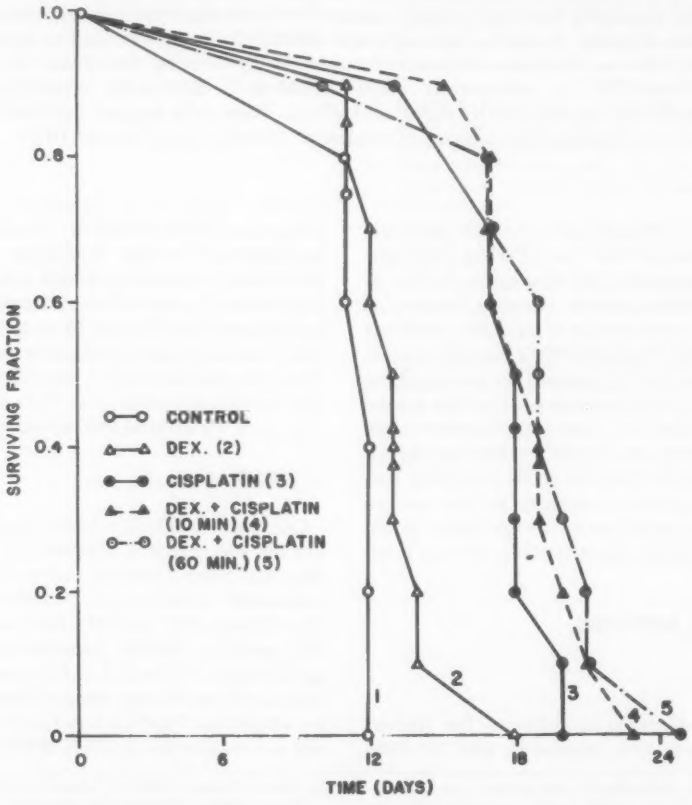

FIGURE 1.—Survival plots for DBA/2 mice administered 10^6 P388 leukemia cells. Median survival times are: untreated mice (Plot 1), 12.0 days; mice treated with Dex (Plot 2), 13.0 days; mice treated with cisplatin (Plot 3), 18 days; mice treated with cisplatin followed by Dex (Plot 4), 18.5 days; and mice treated with a combination of cisplatin and Dex premixed for 60 mins (Plot 5), 19 days. Plots 3, 4, and 5 are statistically different ($P < 0.01$ by log-rank) from Plots 1 and 2.

RESULTS

Figure 1 shows the survival curves for the control and treated leukemic mice. Dex treatment did not significantly increase the lifespan of these mice as compared to the controls (median survival, 12 days). The median survival times for mice treated with cisplatin, Dex followed by cisplatin, or premixed cisplatin-Dex were 18, 18.5, and 19.0 days, respectively. All of these survival times are significantly longer than those of untreated mice or those of mice treated with Dex alone ($P < 0.01$ by log-rank). The slightly shorter survival of the cisplatin-treated mice compared to that of Dex-cisplatin-treated mice is not statistically significant.

Figure 2 shows that in vitro exposure to Dex for 1 hour at 0.01, 0.1, and 1 μg/ml did not change the dose-response curve for cisplatin against HEC-1A clonogenic cells (overall $P = 0.29$). Dex alone had no effect on the survival of HEC-1A TCFU as compared to controls.

Figures 3A and 3B show that cisplatin alone and Dex-cisplatin had similar inhibitory effects on the survival of WiDr TCFU. The simultaneous treatment with cisplatin and Dex and the continuous posttreatment exposure of WiDr clonogenic cells to Dex did not significantly alter the inhibitory effects of 1-hour exposure to cisplatin. Furthermore, a 24-hour pre-exposure of WiDr to Dex, followed by cisplatin treatment (continuous or 1-hour, respectively) with or without postincubation with Dex, did not significantly modify TCFU sensitivity to cisplatin (figs 3C and 3D).

It should be noted that WiDR showed a slightly increased TCFU growth in the presence of Dex (ie, 120%) compared to the controls.

DISCUSSION

Dex does not appear to cause any direct inhibition of the cytotoxicity or antitumor effect of cisplatin, either in vivo or in vitro. In mice with P388 leukemia there was no difference in survival with cisplatin or cisplatin plus short-term Dex treatment. The admixture of both drugs in a syringe for 1 hour followed by ip injection was designed to identify the potential for a direct chemical interaction between the two drugs. However, again the results were similar to those obtained with the sequential ip administration of the two drugs.

The in vitro inhibition by cisplatin of either HEC-1A (human endometrial cancer) or WiDr (human colon

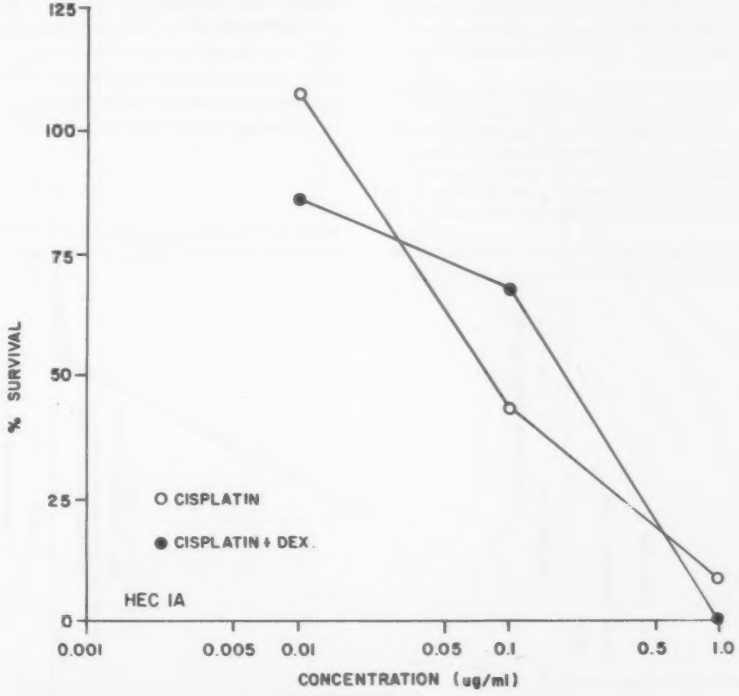

FIGURE 2.—Dose-response of HEC-1A (endometrial) cell line to cisplatin with or without Dex (0.5 μg/ml) ($P = 0.29$).

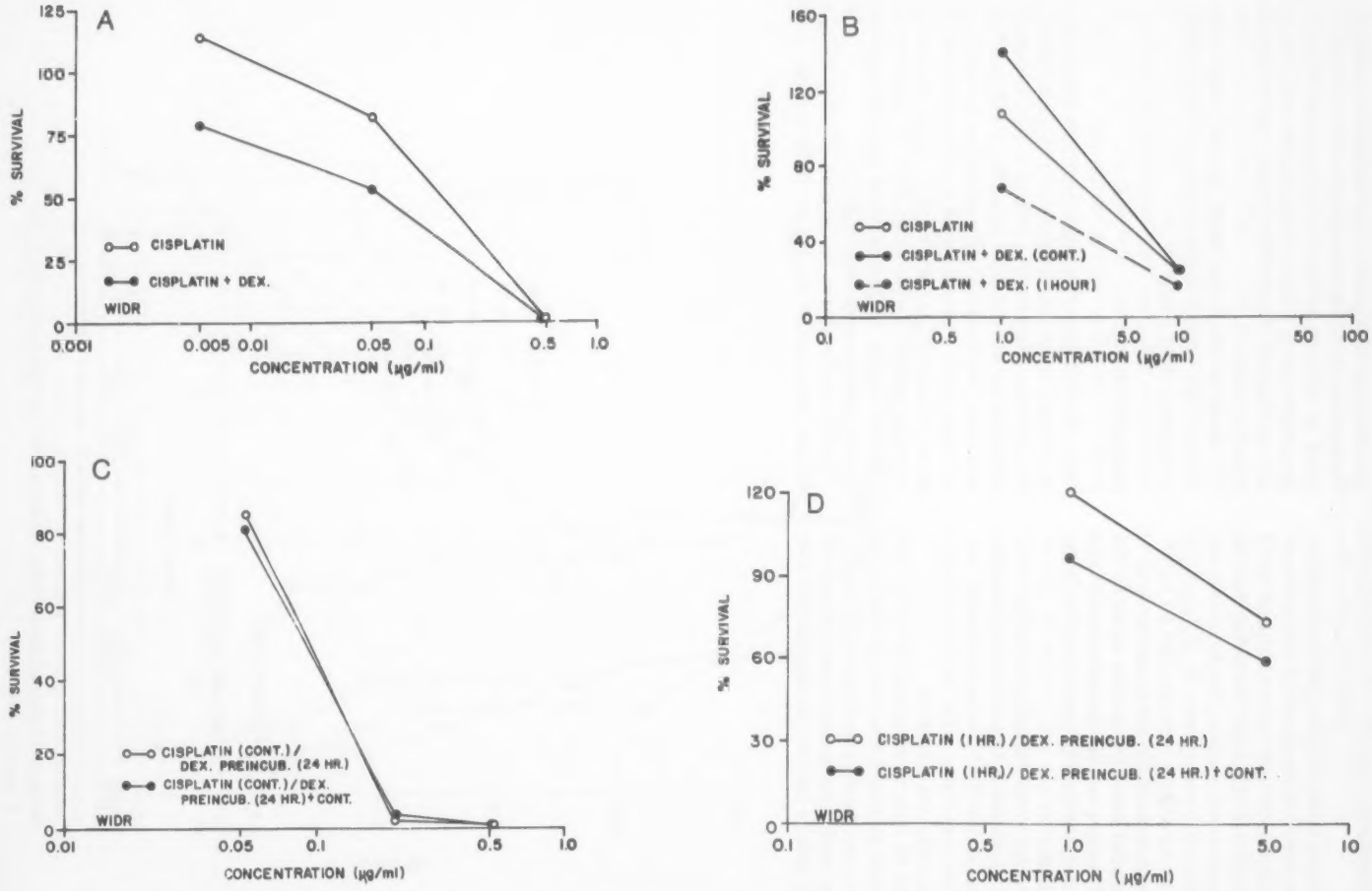

FIGURE 3.—Activity of cisplatin against WiDr (colon) cell line. A. Continuous exposure to cisplatin with (•———•) or without (○———○) 0.5 μg/ml of Dex by continuous, simultaneous incubation. B. 1-hr exposure to cisplatin alone (○———○), cisplatin plus 0.5 μg/ml of continuous Dex (•———•), or cisplatin plus 1 μg/ml of Dex for a 1-hr incubation (•---•). C. Continuous exposure to cisplatin with a 24-hr Dex (0.5 μg/ml) pre-exposure (prior to tumor cell plating) (○———○), or a 24-hr Dex (0.5 μg/ml) pre-exposure plus Dex (0.5 μg/ml) by continuous incubation simultaneously with cisplatin (•———•). D. 1-hr exposure to cisplatin aftera 24-hr exposure to Dex (0.5 μg/ml) followed (○———○) or not followed (•———•) by Dex (0.5 μg/ml) continuous incubation. There is no statistically significant difference between the dose-response curves in any of the experimental sets shown.

adenocarcinoma) TCFU was not significantly influenced by various types of exposure to Dex. Although not statistically significant, the addition of Dex to cisplatin tended to show a slightly additive antitumor effect, ie, increased inhibition of TCFU. This observation is in agreement with a report showing enhancement of cytotoxic DNA cross-linking and phosphorylation of nuclear proteins by alkylating agents when combined with prednisolone (11).

The in vitro concentrations of cisplatin used in these experiments were chosen according to the sensitivity characteristics of the cell lines and human pharmacokinetic data concerning plasma concentrations achieved in vivo after standard doses. The peak plasma concentration of non-protein-bound cisplatin in humans is about 2.5 μg/ml after a dose of 100 mg/m^2 administered over about 1.5 hours (12). The dose of Dex used in these in vitro and in vivo experiments was, of course, calculated on the basis of the doses used in vivo to control emesis in cisplatin-treated patients (1,13,14).

Our data suggest that there is no direct inhibitory effect of Dex on the anticancer activity of cisplatin. Chronic treatment with high-dose glucocorticosteroids does cause immunosuppression and thus might decrease the in vivo antitumor activity of cisplatin and other chemotherapeutic drugs (15). However, it is well-established that the interactions of glucocorticosteroids in antitumor therapy are very complex (16), and a beneficial role of a 1-day, high-dose Dex treatment can not be ruled out. The present clinical use of intermittent, short-duration, high-dose Dex therapy for the control of nausea and vomiting should not affect the therapeutic activity of cisplatin.

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Effect of Temperature on the Cytotoxicity of Vindesine, Amsacrine, and Mitoxantrone[1,2]

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The effect of elevated temperature on the cytotoxicity of three new anticancer drugs (vindesine, mitoxantrone, and amsacrine [AMSA]) was tested in Chinese hamster ovary cells in vitro. Three distinct patterns of interaction with hyperthermia were observed. Vindesine, tested at 37°C, produced a 50% cell kill when concentrations of ≥ 1.0 μg/ml (up to 3.0 μg/ml) for 1 hour were used. At 42.4°C, concentrations > 1.0 μg/ml for 1 hour caused a 60% cell kill (an additive cytotoxic effect). Mitoxantrone produced concentration-dependent lethality at 37°C (89% cell kill after 0.25 μg/ml for 1 hour; 99% cell kill after 1.0 μg/ml for 1 hour). Exposure to mitoxantrone at 42.4°C resulted in synergistic cytotoxicity (97% cell kill after 0.25 μg/ml for 1 hour; 99.98% cell kill after 1.0 μg/ml for 1 hour). In contrast, treatment with AMSA at 42.4°C inhibited cytotoxicity (99.98% cell kill after 5 μg/ml for 1 hour at 37°C; 91% cell kill after 5 μg/ml for 1 hour at 42.4°C). AMSA was not inactivated after being heated at 42.4°C for 1 hour prior to treatment of cells at 37°C. [Cancer Treat Rep 67:1019–1022, 1983]

Hyperthermia has been combined with chemotherapeutic drugs to treat disseminated cancers (1–4). The combination is also being investigated for potential use in localized tumors. Most of the drugs that have been employed clinically with hyperthermia were chosen because of in vitro studies that demonstrated increased cytotoxic effects at elevated temperatures (5). To date, however, clinical results have been somewhat disappointing. This may be due in part to our failure to define the optimum drugs to use with hyperthermia.

Vindesine, mitoxantrone, and amsacrine (AMSA) are three new chemotherapeutic drugs that have demonstrated antitumor effects in human malignancies (6–8). We have determined the effect of elevated temperature on the cellular lethality of these new drugs in order to screen them for possible clinical use with hyperthermia.

METHODS

Cell culture.—Chinese hamster ovary (CHO) cells originally obtained in 1974 from Dr. Ronald M. Humphrey, University of Texas Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, were grown as previously described (9). All experiments were conducted with exponentially growing cell cultures. Doubling time was 13–14 hours; all cell numbers were determined with an electronic particle counter (Model ZBI, Coulter Electronics Inc, Hialeah, FL).

Cell viability.—Cellular survival was determined by the ability of single cells to form colonies as previously described (9). Colony-forming efficiency of control cultures varied between 85% and 95%. The results described represent the means of at least three replicate experiments.

Temperature treatments.—Exponentially growing cell cultures were immersed in water baths maintained at 42.4°C or 37°C by a Haake Model FK water heater. Cell cultures were fully submerged in T_{25} or T_{75} flasks (Falcon Plastics, Oxnard, CA) containing 5 or 15 ml, respectively, of fresh medium. Temperatures quoted are those of the cell monolayer positions and were determined with Thermistor probes.

Drug treatments.—All drugs were prepared fresh before each experiment by dissolving the drug in sterile water. Appropriate dilutions of drug were then added to the cell cultures so that desired drug concentrations could be achieved without the drug solution's exceeding 20% of

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the total volume of solution covering the cellular monolayer. All drug exposures were for 1 hour. Following treatment, cells were washed twice with Puck's saline A (Grand Island Biological Co) and trypsinized (0.05% for 2–3 minutes). After a single-cell suspension had been made, known numbers of cells were plated for colony formation.

RESULTS

Figure 1 shows the cytotoxicity of a 1-hour exposure to various concentrations of vindesine at 37°C and 42.4°C. At 37°C, concentrations > 1.0 μg/ml for 1 hour (up to 3.0 μg/ml) produced similar lethality. Approximately 50% of cells were killed by ⩾ 1.0-μg/ml concentrations of vindesine at 37°C. At 42.4°C, an additive cytotoxic interaction between hyperthermia and vindesine was apparent. Once again, cell survival was unaltered when drug concentrations were increased to > 1.0 μg/ml. At 42.4°C, total cell kill at ⩾ 1.0 μg/ml was approximately 60%. Approximately a 25% cell kill was due to exposure to 42.4°C for 1 hour alone (survival plotted on the Y axis).

Figure 2 shows the cytotoxic interaction between temperature of exposure and mitoxantrone concentrations. As can be seen, markedly synergistic lethality was achieved by exposure to drug at 42.4°C as compared with drug exposure at 37°C. After 1.0 μg/ml for 1 hour, for instance, a 99% cell kill was achieved at 37°C, but nearly a 99.99% kill was produced at 42.4°C. The synergistic effect of drug plus hyperthermia on cell survival was concentration-dependent, however, and was less marked at lower drug concentrations. At 0.5 μg/ml, for instance, the difference between cell kill achieved at 37°C and at 42.4°C was only a factor of 10.

Figure 3 shows the cytotoxic effect of AMSA at 37°C and 42.4°C. In this case, cell killing was inhibited when drug exposure occurred at the elevated temperature. At 37°C, the drug was quite toxic to CHO cells, producing approximately a 99.98% cell kill after exposure to 5 μg/ml for 1 hour. In contrast, a 1-hour exposure to this drug concentration at 42.4°C produced only about a 91% cell kill. Inactivation of the drug by exposure to 42.4°C is not the reason for this effect, since, as is shown, when the drug is heated in medium at 42.4°C for 1 hour and then applied to cells at 37°C, cell killing is unaltered.

DISCUSSION

We undertook these in vitro studies to test for hyperthermia potentiation of drug-induced cell killing. We had hoped that all three drugs would be synergistically cytotoxic with temperature elevation. However, this was clearly not true, and we observed three distinct patterns of interaction.

In the case of vindesine, we had postulated that since hyperthermia had been shown to disrupt tubular structures within cells (10), as do the vinca alkaloids (11), this interaction might be synergistically cytotoxic. We found, however, that simultaneous exposure to vindesine and 42.4°C produced only additive lethality. Similar results

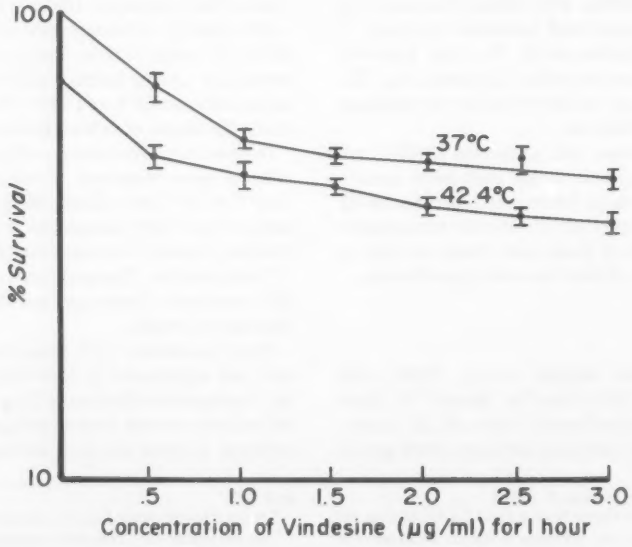

FIGURE 1.—% survival of exponentially growing CHO cells following 1-hr exposure to increasing doses of vindesine at 37°C or 42.4°C. Bars = SE of single-cell survival.

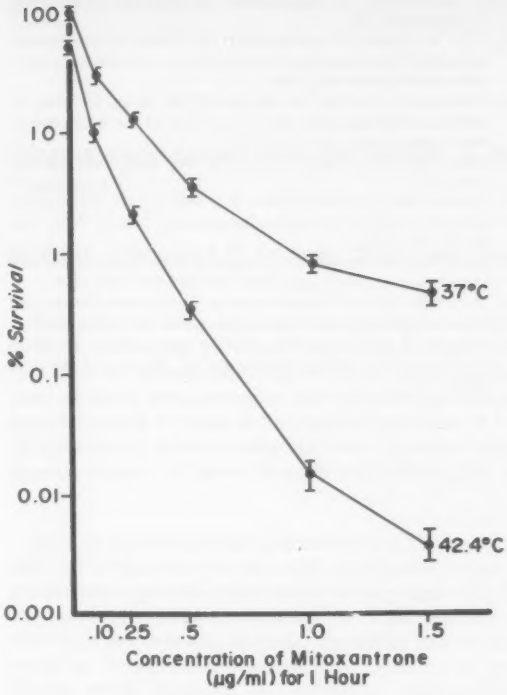

FIGURE 2.—% survival of exponentially growing CHO cells following 1-hr exposure to increasing doses of mitoxantrone at 37°C or 42.4°C. Bars = SE of single-cell survival.

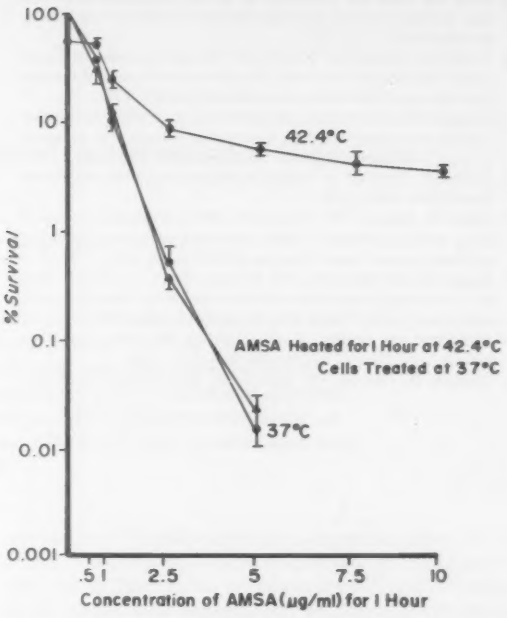

FIGURE 3.—% survival of exponentially growing CHO cells following 1-hr exposure to increasing concentrations of AMSA at 37°C, at 42.4°C, or at 37°C following the heating of AMSA at 42.4°C for 1 hr in medium without cells. Bars = SE of single-cell survival.

with other vinca alkaloids have been recently reported by Hahn (12).

In the case of mitoxantrone, we found a synergistic effect on cell killing when a 1-hour exposure to various concentrations of drug occurred at 42.4°C. This is not surprising since the chemical structure of this drug shares similarities with doxorubicin (13), a drug with in vitro cytotoxicity that is also markedly increased in cells exposed simultaneously to hyperthermia (14).

Finally, in the case of AMSA, we were quite surprised to find reduced cell killing when exposure to the drug occurred at 42.4°C. A similar finding, however, has been described previously, when the cytotoxic interaction between hyperthermia and cytarabine was tested (15).

We have not investigated the possible mechanisms responsible for either the decreased cytotoxicity of AMSA exposure during hyperthermia or the increased cytotoxicity of mitoxantrone at elevated temperature. Other studies (14) have indicated that uptake of doxorubicin (which shares structural similarities with mitoxantrone) is increased at 42.4°C as compared with 37°C; however, it is not now clear whether entry of doxorubicin into cells is necessary for cytotoxicity (16). Antagonism between cytarabine and hyperthermia seems to be the result of altered intracellular drug metabolism at elevated temperature (15). Such an alteration in drug metabolism may account for either the antagonism we found with AMSA or the synergy we observed with mitoxantrone. If the drug entry or metabolism is not significantly changed by temperature manipulation, however, then the interaction between AMSA or mitoxantrone and its predominant cellular target (presumed to be DNA) may be altered either by changes in the type or quantity of damage produced or by the cell's ability to repair this damage.

Since the cytotoxicity of both mitoxantrone and AMSA is markedly affected by the temperature during drug exposure, the potential exists for useful clinical exploitation of these temperature-related effects. For instance, applications of heat to AMSA extravasations may limit tissue damage, while heating mitoxantrone extravasations is probably contraindicated. In addition, use of mitoxantrone with hyperthermia may increase tumor damage. Appropriate animal studies are necessary to validate these in vitro findings.

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Etoposide-Induced Hepatic Injury: A Potential Complication of High-Dose Therapy[1,2]

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Two cases of toxic hepatitis developing in patients receiving high-dose etoposide (VP-16-213) for refractory germinal neoplasms are described. Each patient received a total cumulative dose of at least 6800 mg/m². Liver function abnormalities, including hyperbilirubinemia, elevated transaminases, and elevated alkaline phosphatase, became clinically apparent approximately 3 weeks following the last dose of VP-16-213. These abnormalities resolved spontaneously without sequelae over 12 weeks. High-dose VP-16-213 may be associated with hepatic injury. [Cancer Treat Rep 67:1023–1024, 1983]

Since its introduction into clinical trials in 1973, etoposide (VP-16-213) has proven to be an effective antineoplastic agent against a wide variety of neoplasms including refractory germinal tumors (1–3). Toxic effects include myelosuppression, alopecia, stomatitis, nausea and vomiting, fever, chills, bronchospasm, hypotension, radiation recall reactions, and peripheral neuropathies (1,2,4). In standard doses, VP-16-213 is not felt to be hepatotoxic (5,6). However, the toxicity of high-dose therapy remains incompletely defined. We report two cases of toxic hepatitis following administration of high-dose VP-16-213.

CASE REPORTS

Patient 1

A 25-year-old male was referred to Vanderbilt Medical Center (VMC) with metastatic choriocarcinoma refractory to vinblastine, bleomycin, and cisplatin (VBP). Pretreatment evaluation included normal liver function studies and abdominal computerized tomographic (CT) scan, an elevated β-human chorionic gonadotropin (β-HCG), and multiple pulmonary nodules on chest CT scan. The patient's bone marrow was harvested and cryopreserved, after which he received two cycles of high-dose VP-16-213 (2400 mg/m²) with autologous bone marrow transplantation as previously described (7). There was a marked (> 90%) reduction in all pulmonary masses and a decrease in the β-HCG value from 450 to 0.7 ng/ml (normal, < 0.5). Three cycles of intermediate-dose VP-16-213 (1200 mg/m²) were subsequently administered (7). Prior to the fifth course of VP-16-213, liver function studies were found to be mildly abnormal, ie, SGOT = 206 IU/ml (0–40) and alkaline phosphatase = 111 IU/ml (40–110). Bilirubin was normal. Three weeks later repeat studies revealed SGOT = 1230 IU/ml, alkaline phosphatase = 900 IU/ml, and bilirubin = 8.6 mg/dl (0.2–1.2). HBsAg, anti-HBs, anti-HBc, anti-HA, and monospot test were all negative. Acute and convalescent cytomegalovirus (CMV) and toxoplasmosis serologies were negative. A liver biopsy revealed diffuse hepatic parenchymal necrosis with scattered Councilman bodies, periportal inflammation consisting of mononuclear cells, neutrophils, and numerous eosinophils, with similar histologic findings in the centrolobular regions. The liver function abnormalities returned to normal over the ensuing 12 weeks. The patient had received a total of 8400 mg/m² of VP-16-213, 7200 mg/m² prior to the onset of liver disease. Two units of packed rbcs and 6 units of pooled donor platelets had been administered during treatment. Trimethoprim-sulfamethoxazole was given only during periods of VP-16-213-induced granulocytopenia. Thiethylperazine was used for treatment-related nausea. However, neither of these medications was administered when jaundice developed.

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Patient 2

A 23-year-old male was referred to VMC with an embryonal cell carcinoma refractory to VBP. Pretreatment evaluation revealed no liver abnormalities by chemistries or abdominal CT scan. Alpha-fetoprotein and β-HCG were elevated at 162 ng/ml (normal, < 3.0) and 0.9 ng/ml, respectively. Two courses of high-dose VP-16-213 were given (7). Both markers normalized. Subsequently, two cycles of intermediate-dose VP-16-213 were given without incidence. Prior to a planned fifth course of treatment and 3 weeks after completing the fourth cycle of VP-16-213 (total dose = 6800 mg/m^2) the patient became icteric. Liver studies included: SGOT = 249 IU/L (7-23), alkaline phosphatase = 211 IU/L (25-80), and bilirubin = 4.7 mg/dl (0.2-1.5). No further VP-16-213 was administered. HBsAg, anti-HBc, and anti-HA were negative, as was a monospot test. CMV and toxoplasmosis serologies were also negative. Trimethoprim-sulfamethoxazole and thiethylperazine had been used during treatment, but the patient was on neither when jaundice developed. Nine units of packed rbcs and 24 units of pooled donor platelets had been given during therapy. Within 12 weeks, liver function studies returned to normal.

DISCUSSION

Incriminating a drug as a hepatic toxin is difficult, especially when dealing with an antineoplastic agent (5,6). There are many factors which may cause or contribute to hepatic dysfunction in the cancer patient, including: (*a*) progression of cancer within the liver (5); (*b*) viral or bacterial infection (8); (*c*) possible hepatotoxicity of concomitantly administered therapies, both antineoplastic and supportive (eg, antibiotics); (*d*) possible derangement of liver function through a graft-versus-host reaction after rbc and component transfusions (9); and (*e*) recrudescence of a latent viral infection caused by the antineoplastic therapy (5,10). To establish the hepatic toxicity of an antineoplastic agent, it is necessary to exclude all these causal or contributing factors.

Despite the marked liver function abnormalities, both patients were asymptomatic and afebrile when jaundice developed. Neither appeared to have hepatitis B or A infection, in view of the negative HBsAg, anti-HBs, anti-HBc, and anti-HA. Also, serology tests appear to rule out Epstein-Barr, CMV, and toxoplasmosis infections. Neither patient was septic. Histologically, the liver biopsy was compatible with a drug-induced toxic hepatitis. While both patients had taken trimethoprim-sulfamethoxazole and thiethylperazine, known hepatotoxins (11), neither patient was taking either drug at the time hepatitis developed. These drugs seem unlikely culprits since the incidence of sulfonamide-induced hepatitis is low and usually abrupt in onset (11), trimethoprim is not hepatotoxic (11), and the biopsy was not compatible with the cholestatis usually seen with phenothiazine-induced hepatic injury.

Because of its poor water solubility, VP-16-213 requires packaging in Tween 80, polyethylene glycol 300, anhydrous citric acid, and absolute alcohol (2). Only alcohol is a known hepatic toxin, but in the amount given, it seems unlikely to be responsible for the injury described. The possibility that one of the other solvents might be responsible for the hepatic injury cannot be excluded although there are no reports incriminating these agents.

We know of no report implicating VP-16-213 as a cause of hepatic injury when given in standard doses. Recently, however, Van Echo et al (12) noted transient and reversible SGOT, alkaline phosphatase and bilirubin elevations in two of 12 patients given between 1000 and 1500 mg/m^2 of VP-16-213 for relapsed acute leukemia. We have noted similar abnormalities in some of our patients receiving intermediate- to high-dose VP-16-213 (1200-2400 mg/m^2) (unpublished observations).

Thus, we conclude that VP-16-213, when administered in high doses, may be associated with hepatic injury, either as a consequence of the drug itself or perhaps due to one or more of the solvents in which the agent is packaged. Total cumulative dose may be more important than repeated high individual doses. Careful monitoring of liver function studies during high-dose VP-16-213 therapy is encouraged.

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Fatal Acute Respiratory Failure Following Intrathecal Methotrexate Administration[1]

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A recent report (1) details what is believed to be the first two cases of acute respiratory distress following the administration of methotrexate into the cerebrospinal fluid. We wish to confirm this recent report and to detail the clinical and autopsy findings in a patient with nasopharyngeal lymphoma who developed acute respiratory failure following the intrathecal administration of methotrexate.

CASE REPORT

A previously healthy, 59-year-old, white male presented to the University of Iowa Hospitals with a 1-month history of frontal headaches and blurred vision. Physical examination was remarkable only for proptosis of the left eye. A computerized axial tomographic scan showed a large soft tissue mass involving the paranasal sinuses and left retro-orbital tissues but without direct extension to the brain parenchyma. The ventricles appeared normal and there was no evidence of increased intracranial pressure. Biopsy of the mass revealed a malignant lymphoma (nodular and diffuse, mixed cell type). A spinal tap was performed with the following results: protein, 119 mg/dl; glucose, 35 mg/dl; and cell count, 80/μl. Cytologic examination documented leptomeningeal involvement. Routine laboratory tests including cbc, uric acid, and hepatic and renal functions were normal.

The patient was started on a program of radiation therapy to the tumor and brain (150 rads/daily fraction) and intrathecal methotrexate (15 mg in 15 ml of preservative-free normal saline). Rapid improvement ensued and he was discharged, taking only allopurinol to continue radiation therapy as an outpatient. Two weeks following the initiation of therapy he was feeling well, and a physical examination was normal with the exception of a mild residual proptosis. Examination of the cerebrospinal fluid revealed the following: protein, 72 mg/dl; glucose, 53 mg/dl; and cell count, 18/μl. A third dose of intrathecal methotrexate was administered without difficulty, and the patient returned home. Eight hours later he complained to his family of being unusually fatigued. Over the next 2 hours he became increasingly dyspneic, and 10 hours following the intrathecal therapy a family member found him diaphoretic and in marked respiratory distress, with the production of "pink-tinged sputum." Cardiopulmonary arrest ensued and, despite resuscitative efforts at home and upon arrival at the hospital, the patient died shortly thereafter.

At autopsy the body was that of a thin, healthy-appearing male. Scattered foci of residual lymphoma were present in the frontal and ethmoid sinuses and in the soft tissue of the left orbit. The infiltrate in these areas consisted of small, 20–30-cell aggregates of lymphoid cells identical to those seen in the previous biopsy. A single focus of dense lymphomatous infiltrate was present in the left kidney. The meninges showed occasional, small, 10–15-cell lymphoma cell aggregates. No infiltrates were seen in the brain parenchyma and there was no evidence of ventricular enlargement or other abnormalities.

The lungs weighed 405 and 480 g. Frothy fluid was easily expressed from the cut surface of both lungs. Microscopically, there was atelectasis and many areas of intra-alveolar hemorrhage. Hemosiderin-laden macrophages were numerous and intra-alveolar collections of edema fluid were noted. Postmortem lung and blood cultures were negative.

Extensive gross and microscopic examination of the heart failed to reveal any evidence of myocardial damage. Coronary atherosclerosis was mild (< 10% occlusion in all main arteries), and the left ventricular thickness was normal.

DISCUSSION

Bernstein et al (1) recently described two children who developed pulmonary edema following the administration of methotrexate into the cerebrospinal fluid. The occurrence of pulmonary edema secondary to methotrexate administration is unusual. The most commonly reported

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pulmonary complication of methotrexate therapy is an interstitial pneumonitis (3) occurring 12 days to 5 years after initiation of therapy. The route of drug administration is usually oral, although pneumonitis has been reported after both iv and intrathecal administration (4,5). At the present time the pneumonitis is suspected to be due to a direct toxic effect of the drug or to some as yet unknown immunologic hypersensitivity mechanism. Histologically, there is an interstitial and alveloar mixed cell infiltrate with or without a granulomatous component. Interstitial fibrosis has also been reported.

In contrast to this often self-lin.ited pneumonitis, Lascari et al (6) described an 11-year-old girl who died suddenly as a result of acute pulmonary edema following her first oral dose of methotrexate. In addition, two cases of anaphylactoid-type reactions in patients receiving high-dose iv methotrexate have also been reported (7).

Our case clearly differs from the typical case of methotrexate-induced pneumonitis. The rapid onset of respiratory distress with the production of frothy sputum and the pathologic findings at autopsy indicate acute pulmonary edema as the cause of death. While it is possible that the pulmonary edema was cardiogenic in origin, this seems to be unlikely considering the negative cardiac findings at autopsy and the lack of predisposing factors for heart disease, ie, severe coronary atherosclerosis or hypertension. A more likely possibility in view of their temporal relationship would be that the intrathecal administration of methotrexate was in some manner related to the onset of a neurogenic pulmonary edema.

The exact mechanism by which neurogenic pulmonary edema arises is unknown. According to the theory proposed by Theodore and Robin (8,9), injury or some other stimulus results in a centrally mediated massive sympathetic discharge.

The mechanism by which the intrathecal use of methotrexate could result in neurogenic pulmonary edema is unknown and the need for experimental data is obvious. As postulated by Bernstein et al (1), it is possible that the methotrexate has a direct toxic effect on the hypothalamus with the release of a massive centrally mediated sympathetic discharge leading to fluid shifts in the pulmonary circulation.

We have presented the clinical and pathologic details of what is believed to be a case of neurogenic pulmonary edema secondary to the injection of methotrexate into the cerebrospinal fluid. This is a newly recognized complication of methotrexate therapy and requires further substantiation to determine its incidence and pathogenesis.

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Phase I Trial of PCNU[1,2]

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In a phase I trial, 28 patients with advanced solid tumors were given PCNU daily for 5 days by iv infusion over 20 minutes at 6-week intervals. The total dose for each course ranged from 25 to 150 mg/m² in six escalation steps. Myelosuppression was dose-limiting, with a platelet count depression regularly observed at doses of ≥ 82.5 mg/m². Leukopenia occurred only at higher doses. Nausea was uncommon, and vomiting did not occur. There were no adverse drug-related effects on renal or hepatic function. No antitumor activity was observed. A dose range of 100–125 mg/m² iv for each 5-day course (20–25 mg/m²/day) is recommended for phase II studies. [Cancer Treat Rep 67:1027–1029, 1983]

PCNU is an iv administered lipid-soluble nitrosourea. In a structure-activity study of nitrosoureas in the Lewis lung carcinoma system, PCNU had optimal lipophilicity which predicted for maximal activity in this system (1). Studies using L1210 leukemia also predicted an advantage for PCNU, for it had the lowest carbamoylating and highest alkylating activities of the available nitrosoureas (2), properties correlating with activity in this model (3).

PCNU has demonstrated antitumor activity in several tumor systems, including Lewis lung carcinoma, B16 melanoma, L1210 leukemia, and P388 leukemia. It has also shown significant activity against several intracranially implanted tumors, including L1210 leukemia and murine ependymoblastoma. This activity was better than that of carmustine, semustine, chlorozotocin, and streptozocin; it was equivalent to that of lomustine (2). Toxicology studies in large animals reported that myelosuppression, vomiting, diarrhea, and elevation of hepatic enzymes were prominent effects.

Carmustine, lomustine, and semustine are usually administered every 6 weeks in single doses which are often associated with significant nausea and vomiting. A study using semustine in combination with 5-FU in colon cancer has shown lessened nausea and vomiting, with no loss of efficacy, when this nitrosourea is given in divided doses as opposed to a single administration (4). Based on this observation and on the lack of schedule dependency in preclinical trials (2), a schedule of daily administration on each of 5 consecutive days was selected for this phase I trial of PCNU.

PATIENTS AND METHODS

Patients with histologic evidence of advanced malignancy for whom standard therapy was not indicated were included in this study. Each patient had a Karnofsky performance status of ≥ 50 and an estimated survival of at least 8 weeks. No patient had received other therapy for at least 28 days prior to receiving PCNU. All patients had acceptable hematologic status (wbc count > 4000/mm³ and platelet count > 100,000/mm³) and normal hepatic and renal functions.

PCNU was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. PCNU, diluted with 5% dextrose to a concentration ≤ 0.5 mg/ml, was given by slow iv administration (over 20 minutes) in equal doses for 5 consecutive days. Courses were repeated every 6 weeks. This trial was initiated at a total dose level of 25 mg/m²/course (5 mg/m² iv daily × 5). Escalation proceeded using doses of 50, 82.5, 100, 125, and 150 mg/m²/course. At least three patients were entered at each dose level. At any level at which toxicity was seen, at least four patients were entered. Standard response criteria were used.

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TABLE 1.—PCNU toxicity in 28 patients

Total dose/ 5-day course* (mg/m^2)	No. of treatment courses	Wbc count nadir			Platelet count nadir		
		Median ($\times 10^3$ cells/mm^3)	Range	Median Day	Median ($\times 10^3$ cells/mm^3)	Range	Median Day
25	3	5.2	3.7-5.9	44	237	130-298	12
50	3	4.8	3.0-4.9	33	200	180-232	29
82.5	4	4.0	2.9-4.0	29	101	19-135	30
100	8	5.8	1.4-10.9	41	74	12-175	34
125	13	3.4	1.3-5.2	37	50	14-225	30
150	8	2.8	0.5-5.5	37	28	10-120	31

* Drug administered in 5 equal daily doses.

Prior to the start of therapy, all patients had a complete history and physical examination and a laboratory evaluation including cbc with differential, 12-channel screening profile, 5'nucleotidase, serum electrolytes, prothrombin time, serum creatinine, urinalysis, and ECG. Biochemical and hematologic studies were repeated weekly.

The median age of patients was 56 years (range, 27-74). There were 16 men and 12 women. Karnofsky performance status was 70, 80, or 90 in 17 patients and 50 or 60 in 11. Prior treatment included chemotherapy (nine patients), radiotherapy (ten), chemotherapy plus radiotherapy (six), and none (three). Twenty-three patients had non-small cell lung cancer (21 with adenocarcinoma, one with epidermoid carcinoma, and one with large cell carcinoma), three had adenocarcinoma of unknown primary, one had renal cell carcinoma, and one had glioblastoma.

RESULTS

As outlined in table 1, thrombocytopenia was the most frequently observed hematologic effect. Median platelet count nadirs < 100,000/mm^3 were observed at all dose levels > 82.5 mg/m^2/course. No serious hemorrhage occurred; however, five patients required platelet transfusions. At higher dose levels, thrombocytopenia was maximal at 4-5 weeks, occurring approximately 1 week earlier than the lowest leukocyte count. Recovery occurred between Days 42 and 50. Six patients received a second course of PCNU at the same dose level. All patients had lower platelet counts during the subsequent course. A median wbc count nadir of < 4000/mm^3 was observed only with PCNU doses of ≥ 125 mg/m^2.

Only ten of 39 courses were accompanied by nausea, and no patient experienced vomiting. There were no instances of phlebitis or skin ulceration at injection sites. One patient with adenocarcinoma of the lung, with a performance status of 90 and a normal neurologic examination, experienced a transient episode of aphasia immediately following drug administration at 150 mg/m^2. Neurologic examination, EEG, and a computerized tomographic scan of the brain were normal.

Twenty-eight patients received 39 courses of PCNU. Among 14 patients with measurable lesions, there were no objective antitumor responses.

DISCUSSION

PCNU was chosen for clinical trials because of its optimal lipophilicity, high alkylating activity, and preclinical antitumor activity. Preliminary pharmacokinetic evaluations indicate penetration of the drug into the cerebrospinal fluid (5). In this phase I study of PCNU, myelosuppression was dose-limiting. PCNU produced consistent thrombocytopenia when doses ≥ 100 mg/m^2/course were administered. Higher dose levels produced a more marked decrease in the median platelet count nadir. Leukopenia occurred at the highest dose levels examined. Greater myelosuppression was observed in six patients given repeat doses at the same level, suggesting cumulative myelosuppression with PCNU. These findings were consistent with other phase I studies (6-9).

The daily × 5 schedule produced little immediate toxicity. The findings of mild hypotension (7) and the syndrome of anorexia with malaise (6) observed in other trials were not encountered. Based on this phase I trial, we suggest a phase II single-agent administration of PCNU at 100-125 mg/m^2 iv divided over 5 days. Because cumulative myelosuppression, especially thrombocytopenia, may occur, caution should be used in escalating the dose of this agent in subsequent treatment cycles.

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Iv Administration of a Water-Insoluble Antimitotic Compound Entrapped in Liposomes. Preliminary Report on Infusion of Large Volumes of Liposomes to Man[1,2]

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Five patients with advanced multiresistant neoplasms were infused with NSC-251635 (a water-insoluble antimitotic agent) entrapped in egg yolk lecithin, cholesterol, and stearylamine liposomes. The maximum volume injected was 400 ml containing 8 g of lipids, ie, a dose of 135 mg/kg of body weight. Overall tolerance of the treatment was excellent. This study indicates that liposomes may be used safely as carriers for the iv administration of water-insoluble drugs to humans. [Cancer Treat Rep 67:1031–1033, 1983]

Since potentially useful therapeutic agents cannot be tested in man because of their hydrophobic properties, it occurred to us that entrapment into liposomes could allow their injection by the iv route. We studied the effects of NSC-251635, a water-insoluble antimitotic quinazolone derivative, in L1210 murine leukemia. After entrapment into liposomes, the compound could be safely injected into mice by the iv route and showed a therapeutic activity against the ip and the iv L1210 leukemias (1). The liposomal preparations were endotoxin-free and could be microbiologically sterilized by megavoltage radiation without alteration of their structure and decrease of their therapeutic efficacy (1).

MATERIALS AND METHODS

Only patients with advanced neoplastic disease resistant to conventional therapy were admitted to the study. Informed consent was obtained. Each patient had satisfactory renal, hepatic, cardiopulmonary, and hematologic (for the solid tumors) functions. 2-[3′-(Methoxycarbonylamino)-phenyl]-3-phenyl-6-methoxycarbonylamino-4-(3*H*)-quinazolone (NSC-251635) was entrapped in liposomes made of egg yolk lecithin, cholesterol, and stearylamine at the molar proportion of 4:3:1 (2). The lipid concentration was 20 mg/ml. The diameter of the lipid vesicles ranged from 30 to 110 nm, as determined by negative-stain electron microscopy (1). Concentration of NSC-251635 entrapped in the liposomes ranged from 625 to 1000 μg/ml, with a median value of 706 μg. All liposomal preparations were free of endotoxin, as demonstrated by the limulus test. Before the infusion, each preparation was irradiated with a 1.50-Mrad dose administered by an irradiator loaded with a ^{60}Co source of about 18,000 Ci (666 TBq). The sterility of each irradiated liposomal preparation was demonstrated by the microbiologic tests.

Taking into account the dose of NSC-251635 active in mice bearing L1210 leukemia (1), the dose presumed to be active in man was estimated to be 2 mg/kg of body weight (3). The first patient received this dose entrapped in a 210-ml liposomal preparation; during the following infusions, progressively higher volumes of liposomes were injected.

Administration of the preparation was performed in the intensive care unit of the Department of Medicine, Institut Jules Bordet, where the patients were carefully monitored for side effects, vital parameters, ECG, and blood and urine chemistries including coagulation, he-

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TABLE 1.—Patient characteristics and treatment regimens

Patient No.	Sex/age (yrs)	Body surface area (m^2)	Body weight (kg)	Type of neoplasm	Evolutive site	Infusions				Interval between 2 courses (days)	Survival (mos)
						1st course		2nd course			
						Volume (ml)	Drug (mg)	Volume (ml)	Drug (mg)		
1	M/52	1.70	61	NSCLC*	Brain	210	139	296	222	42	7
2	M/55	1.50	50	NSCLC*	Brain	248	173	310	261	50	3
3	M/63	1.80	68	NSCLC*	Brain Lung	305	207	—	—	—	1
4	M/44	1.65	59	Adenocarcinoma, primary unknown	Lung Skin	300	300	400	250	29	3
5	F/31	1.52	52	Erythroleukemia	Bone marrow	225	158	205	160	7	4

* Non-small cell lung carcinoma.

molysis, arterial gas, and complement system tests. An EEG record and a chest x-ray film were obtained before and 24 hours after the start of the infusion. In one patient (No. 4), an EEG record was taken 1 hour after the start of the infusion.

To study biodistribution, three patients were also injected iv with a 5-ml bolus of liposomes labeled with $^{99m}TcO_4$ (final radioactivity, 18–20 mCi or 666–740 MBq) (4). One patient received drug-free liposomes and the other two patients received liposomes containing NSC-251635.

RESULTS

Five patients, four with solid tumors and one with erythroleukemia, were admitted to the study (table 1). Four patients received a second course of treatment. The highest liposomal volume administered so far has been 400 ml containing 8 g of lipids, ie, a dose of 135 mg/kg of body weight. The highest dose of NSC-251635 injected has been 300 mg.

All infusions were tolerated by the patients. The only clinical side effect observed was a transient drowsiness that occurred during the first hour of the infusion and cleared within 3 hours. No modification of the EEG was noted during or after the liposome infusion. Little change was observed in the blood parameters. An increase in the serum phospholipid content, ranging from 35% to 77% (average, 49%) of the initial level, occurred 2–3 hours after the start of the liposome administration and returned to the preinfusion value within the first 24 hours. Activation of the complement system occurred in the first 6 hours following the start of the infusion, as shown by a constant increase in the complement components C3d/C3 ratio.

No objective regression of tumor tissue in the patients with a solid neoplasm and no modification of the course of the erythroleukemia were observed. The major site of uptake of both labeled drug-free liposomes and liposomes containing NSC-251635 was the liver (17%–36% of total radioactivity of the body, 24 hours after the injection).

DISCUSSION

Only a few studies dealing with the iv administration of liposomes in man have been reported so far, and they relate to the administration of small volumes of liposomes containing a water-soluble compound (5). The infusions of liposomes containing NSC-251635, a water-insoluble antimitotic compound, were very well tolerated, although administered in a large volume of liposomes. All patients experienced mild sedation, perhaps induced by NSC-251635, which belongs to a class of drugs with hypnotic and tranquilizing properties. The observed increase in serum phospholipid level suggests the persistence of the liposomes in the blood circulation during at least 24 hours, which may explain the small uptake observed in an organ such as the spleen (6). The activation of the complement system was not associated with clinical manifestations. As indicated by the results of the present study, uncoated liposomes may constitute an adequate carrier system for the introduction of water-insoluble drugs by the iv route in man. The lack of toxicity observed with the reported liposome volumes and drug doses allows us to continue our study with higher volumes and repeated courses of liposomes.

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Cerebrospinal Fluid-Platinum Kinetics of Cisplatin in Man[1]

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A pharmacokinetic analysis of total platinum has been conducted in plasma and cerebrospinal fluid (CSF) of a patient who received an iv bolus injection of cisplatin at a dose of 80 mg/m^2 for two courses separated by 4 weeks. The platinum elimination from plasma was characterized by two phases, with half-lives of 8.1 mins and 95.8 hrs for the first course and 25.1 mins and 91.0 hrs for the second course. CSF platinum depletion followed a single exponential, with half-lives of 43.5 and 51.1 mins, respectively, for the two successive courses. The peak CSF platinum concentration (~0.5 μM Pt) was reached < 1 hr after the end of the iv bolus cisplatin administration.

Cisplatin is an antineoplastic agent which has demonstrated activity in the treatment of different human tumors, both as a single agent and in combination (1–3). It is widely used in testicular and ovarian cancers and also in head and neck, bladder, prostate, and lung cancers. The clinical results were largely presented in two recent symposia (4,5). The use of cisplatin in the treatment of brain tumors has not yet been well-developed, but preliminary studies are encouraging (6–8). We present the pharmacokinetics of platinum in the CSF and plasma of one patient after a systemic administration of cisplatin during two courses.

MATERIALS AND METHODS

Case Report

The patient was a 41-year-old woman with an inoperable glioblastoma of the cerebellar peduncles diagnosed by computerized tomographic scan and biopsy (Nov 1977). Nine months after external radiotherapy (Jan 1979, 5500 rads in 6 weeks), the patient relapsed. A failure with lomustine treatment confirmed by computerized tomographic scan initiated a phase II trial with cisplatin (Cisplatyl, Roger Bellon, France) for two courses separated by a 4-week iv bolus (15 mins) at a dose of 80 mg/m^2 (140 mg of cisplatin). Hydration was performed 4 hrs before cisplatin treatment with 2 L of saline solution and continued after cisplatin administration with 250 ml of mannitol. The patient received ACTH treatment with constant medical care. This adjuvant therapy is probably responsible for the fast improvement of the general condition of the patient. The only toxic effect experienced was mild emesis. Blood samples were obtained with a heparinized valve permanently introduced in the left arm and CSF samples were aspirated with a 25-gauge needle in the proximal reservoir of the ventriculo-peritoneal valve (Am's valve). Samples were collected just prior to cisplatin administration and at various times: 5 mins, 10 mins, 30 mins, 1 hr, 4 hrs, 8 hrs, 1 day, 1.5 days, 2.5 days, 3.5 days, 4.5 days, 5.5 days, 6.5 days, 7.5 days, 8.5 days, 9.5 days, and 10.5 days following the end of the cisplatin injections. No intracranial hypertension could be detected.

Platinum Analysis

A Perkin-Elmer 603 atomic absorption spectrophotometer coupled with an HGA-76 graphite furnace and an automatic sampler AS-1 were used to quantify the platinum content of the fluids (9). The platinum species in plasma and in CSF were not studied.

Pharmacokinetic Analysis

The platinum concentration in plasma as a function of time after administration of the cisplatin was fitted using an analytic expression for the platinum concentration in plasma consisting of the sum of two exponentials. The data for the CSF are well-represented by a single exponential over the interval up to 1 day. Solutions were obtained using a least-squares fitting routine in MLAB (10).

RESULTS AND DISCUSSION

The results obtained using the fitting routine gave a good fit for the whole sampling time interval for both

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plasma and CSF and are shown in figure 1, where the points denoted by squares and circles represent the experimental values. The values of the half-lives are shown in the figure. The estimate of the error in these constants was derived from the values of the diagonal terms of the matrix used in computing the rate constants.

Just prior to the second cisplatin course, a concentration of 0.54 μM of platinum was measured in plasma and subtracted from the plasma experimental values of the second course, while no platinum could be detected in the CSF after 1.5 days for the first course and after 12 hrs for the second course. The results obtained for the plasma in the present study show two very distinct regimens of depletion of platinum, with half-lives corresponding to 8.1 mins (k_1 = -0.0857 min^{-1}) and 95.8 hrs (k_2 = -0.000121 min^{-1}), respectively, in the first course and 25.1 mins (k_1 = -0.0276 min^{-1}) and 91.0 hrs (k_2 = -0.000127 min^{-1}) in the second course of drug administration. These values are in the range observed by others (11,12). The maximum CSF platinum concentration in the present study was attained between 5 and 30 mins in the first course (0.46 μM Pt) and between 10 and 60 mins in the second (0.49 μM Pt). No attempts have been made to characterize the platinum chemical species in the CSF. The depletion of platinum from the CSF is well-described by a single exponential, with a corresponding half-life of about 43.5 mins (k = -0.01592 min^{-1}) and 51.1 mins (k = -0.01355 min^{-1}), respectively, for the two successive courses.

Some objective responses in brain tumors have been reported with the use of cisplatin either alone (6,7) or in combination with bleomycin and vinblastine (8). The pharmacokinetic behavior of cisplatin and platinum analogs has been reported in CSF of rhesus monkeys (13). CSF maximum concentrations of 0.35 and 0.78 μM platinum were obtained with iv bolus injections of 1.5 and 3.0 mg/kg of cisplatin, 30–40 minutes following the cisplatin administration, the ratio CSF-Pt/plasma-Pt never exceed-

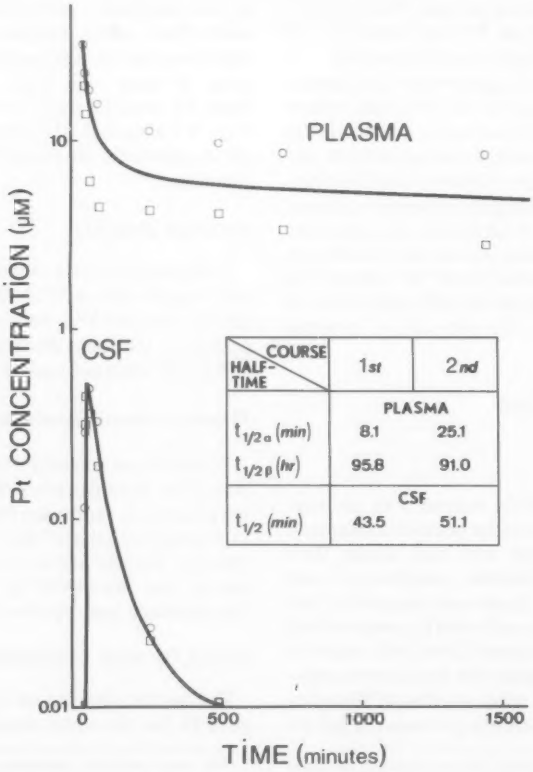

FIGURE 1.—Pharmacokinetics of cisplatin in plasma and CSF. □ and ○ = the experimental values obtained during the 1st and 2nd course, respectively. The inset indicates the half-lives of platinum elimination. Not all of the data points (from 5 mins to 10.5 days) used in the analysis are shown.

ing 4%. The half-life of the platinum elimination from the CSF was about 60 mins. In man, platinum concentrations in the CSF were reported to be barely detectable (< 0.5 μM) but not quantifiable (< 2 μM) using x-ray fluorescence spectrometry (14), which does not seem to be as sensitive a technique as flameless atomic absorption spectrometry for platinum quantitation. The only reported pharmacokinetic study for cisplatin in the CSF in man was achieved during combination chemotherapy treatment with bleomycin and vinblastine (8). The peak levels of platinum in the CSF were reached about 30 mins after the cisplatin administration and the half-life of disappearance of platinum from the CSF was cited as 1.44 (± 0.12 SD) hrs.

The results of this study clearly show and confirm the fact that a nonnegligible amount of platinum is found in the CSF. It is now of interest to determine what the platinum species are, free cisplatin or protein-bound platinum complexes, and if they preserve antitumoral activity.

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Phase II Study of Mitoxantrone in Patients With Metastatic Breast Carcinoma: A Southeastern Cancer Study Group Project[1,2]

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The anthracenedione compounds have been found to have significant activity in experimental tumor systems. Mitoxantrone has induced both a significant increase in lifespan and long-term survival in several animal tumor models (1,2). The Southeastern Cancer Study Group (SECSG) performed a phase II study in patients with metastatic breast cancer, using a weekly schedule developed in previous phase I trials (3,4). All patients had been previously treated with chemotherapy including doxorubicin.

Eligibility requirements included progressive stage IV disease; demonstrable resistance to a doxorubicin-containing combination (or the attainment of a maximum tolerable cumulative dose, ie, 450 mg/m^2 of doxorubicin); resistance to standard hormonal treatment or estrogen-receptor-negative tumor; and normal renal, hepatic, and bone marrow functions (no level comparable to grade 1 toxicity or worse). Mitoxantrone was supplied by Lederle Laboratories and was administered weekly for 6 weeks at a dose of 5 mg/m^2 by a 30-minute iv infusion. Standard response and toxicity criteria of the SECSG were utilized (5).

Forty-two patients were entered (table 1). Thirty-one patients were evaluable for response; of these, two had a partial response (duration, 4 and 5 months), for a response rate of 6.5% ± 1%–21% (± 1 SD). Seven evaluable patients remained stable and 22 had disease progression. Neither responding patient had a tumor demonstrably resistant to doxorubicin. Neutropenia was dose-limiting. Gastrointestinal toxicity was minimal. Five patients developed cardiac disorders, four having previously received 450 mg/m^2 of doxorubicin. Two patients developed congestive cardiac failure after receiving 80 and 100 mg/m^2 of mitoxantrone. The ejection fractions of two other patients decreased to 44% and 42% after 5 and 6 weeks of mitoxantrone. One patient (previous doxorubicin dose, 215 mg/m^2) died suddenly 1 week after receiving his 12th weekly dose of mitoxantrone.

This phase II study has demonstrated that mitoxantrone may induce a brief response in previously heavily treated patients with advanced breast cancer. Gastrointestinal side effects are virtually nonexistent and neutropenia is readily managed by dose adjustment. Additionally, it appears quite likely that mitoxantrone can potentiate doxorubicin cardiac damage.

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[5]We express our appreciation to Theresa Logan and Randi Sigal for help with data management.

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TABLE 1.—Patient characteristics

	No. of patients	
	Evaluable	Inevaluable
Total	31	11*
Age in yrs		
< 50	13	5
> 49	18	2
Performance status		
50%-60%	10	4
70%-80%	13	2
90%-100%	8	1
Estrogen-receptor status		
Positive	9	1
Negative	10	2
Unknown	12	4
Prior treatment with doxorubicin		
Resistant	25	7
Total dose = 450 mg/m^2	6	0
No. of prior chemotherapy regimens		
1	9	1
2	11	0
3	5	6
⩾ 4	6	0
Sites of disease (patterns of metastases)		
Primary tumor	2	0
Bone only	0	2
Chest wall, nodal	11	1
Pleural	7	1
Pulmonary, nodular-lymphangitic	4	2
	3	0
Hepatic/widespread	4	1
No. of sites of metastases		
1-2	15	4
3-4	16	3
Toxicity		
Neutropenia (cells/mm^3)		
None	4	3
< 2000	3	0
< 1500	11	2
< 1000	6	1
< 500	7	2
Gastrointestinal		
None	23	7
Moderate nausea/vomiting	8	1
Cardiac	5	0

* Includes 7 patients with adequate data. Reasons for inevaluability (11 patients) were: inadequate treatment (< 2 doses), 2 patients; protocol violation (sporadic treatment), 1; no measurable disease, 2; neutropenia prior to treatment (received 1 dose—toxicity), 1; concurrent treatment (with prednisone), 1; and no data supplied (registered by phone—inadequate data sheets submitted), 4.

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Phase II Evaluation of Vindesine in Non-Small Cell Bronchogenic Carcinoma[1]

Andrew W. Hutcheon,[2,*] James B. D. Palmer,[2] Michael A. Pratt,[2] and Roland A. Clark[3]

The activity of vindesine in non-small cell lung cancer was identified by Smith et al (1) and confirmed by others (2,3). In addition, it has been shown that patients not previously treated with chemotherapy had a higher response rate than patients who had received prior cytotoxic treatment. Therefore, we found it relevant to confirm the activity of vindesine in patients with non-small cell lung cancer who had not received prior chemotherapy.

PATIENTS AND METHODS

Forty-eight patients were entered in the study. All patients had objectively measurable disease, histologic confirmation of the diagnosis, an Eastern Cooperative Oncology Group (ECOG) performance status of 0–3, and a life expectancy of > 2 months. Ethical committee approval and informed consent were obtained.

Before initiation of treatment, all patients were staged according to the criteria of the Veterans Administration Lung Cancer Study Group (4). Response was evaluated by standard criteria (5), and duration of response was defined as the time from initiation of treatment to disease progression. Patient characteristics, staging, and response to therapy are summarized in table 1.

Vindesine (supplied by Eli Lilly and Co, Basingstoke, England) was given weekly as a bolus injection through a free-flowing iv line at a dose of 3 mg/m^2 for 6 weeks. An adequate trial required a minimum of four weekly doses of vindesine. Patients who responded and those patients with stable disease continued to receive the drug every 2 weeks.

RESULTS

There were no complete responses in any of the groups. Two patients with epidermoid carcinoma, five with adenocarcinoma, and two with large cell undifferentiated carcinoma achieved a partial response. In all responding patients, objective tumor regression occurred within 4 weeks of initiation of treatment. Sites of response were lung (seven patients), lymph nodes (three), pleura (one), and liver (one). Three of the nine patients responded in more than one site. Prior treatment with radiography did not affect the response rate. No major differences in the extent or distribution of disease were observed between responding and nonresponding patients.

The toxicity seen was similar to that reported by others (2,3). The median number of doses given was five per patient (range, four to 29). Neurotoxic effects were most often manifested by nonprogressive paresthesia which were cumulative on the weekly schedule but did not progress on the maintenance schedule. Leukopenia was mild and dose-limiting in only 15% of patients receiving the weekly regimen.

DISCUSSION

This study confirms that vindesine is an active agent in the treatment of adenocarcinoma of the lung, and also shows promise in patients with large cell undifferentiated carcinoma. The 8% response rate in epidermoid carcinoma was disappointing and is in contrast to the rate found by Gralla et al (3). This may be due in part to the scheduling used in this study, and it is unlikely that this particular regimen will prove to be the most appropriate.

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TABLE 1.—Patient characteristics and responses

Characteristic	All patients	Response		
		Partial	Stable	Disease progression
Median age in yrs (range)	62 (43-70)	59 (43-70)	61 (54-70)	57 (46-70)
Males/females	39/9	5/4	13/3	21/2
Median performance status (range)	1.2 (0-3)	1.4 (0-3)	1.3 (0-3)	1.6 (0-3)
No. of patients				
Prior radiotherapy	37	5	19	13
Staging*				
Limited	22	4	8	10
Extensive	26	3	7	16
Weight loss	25	3	5	17
Asymptomatic	9	2	4	3
Histologic type (total)	48	9	16	23
Epidermoid carcinoma	24	2	10	12
Adenocarcinoma	17	5	4	8
Large cell undifferentiated carcinoma	7	2	2	3
Toxic effects				
Neurotoxic effects	31			
Alopecia	27			
Constipation	26			
Nausea or vomiting	22			
Fever (38°-39°C)	11			
Thrombophlebitis	4			
Wbc count nadir (cells/mm^3)				
> 4000	8			
3000-3999	18			
2000-2999	17			
1000-1999	5			
Platelet count nadir (cells/mm^3)				
> 150,000	39			
100,000-149,000	6			
< 100,000	3			
Survival (mos)				
Responders (range)	2-13			
Median	7			
Nonresponders (range)	1.5-7			
Median	2.5			

* Veterans Administration Lung Cancer Study Group criteria.

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Phase II Study of Amsacrine in Metastatic Renal Cell Carcinoma: A Cancer and Leukemia Group B Study[1]

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A new chemotherapeutic agent, amsacrine (AMSA), synthesized by Cain and Atwell (1), has recently passed phase I studies (2,3) and several phase II trials (4,5). It is a member of a new class of antineoplastic agents derived from the three-ring acridine molecule. Its activity results from binding to DNA through intercalation in a manner slightly different from the anthracyclines (6). A wide spectrum of antitumor activity has been found in early clinical studies (4), including responses in patients with adenocarcinoma (2). In 1979, because stage IV renal cell carcinoma has such a poor prognosis (7) and because few drugs of any activity have been found for the disease, the Cancer and Leukemia Group B initiated a phase II study of AMSA in the treatment of patients with metastatic hypernephroma.

PATIENTS AND METHODS

Patient Selection

Between June 1979 and April 1980, 42 patients were entered in this study of AMSA in hypernephroma. All patients were evaluable. Patients were required to have measurable, metastatic, histologically documented hypernephroma, wbc count $> 4000/\mu l$, platelet count $> 100,000/\mu l$, bilirubin < 3.0 mg/dl, SGOT less than three times normal, and alkaline phosphatase less than three times normal. Most patients (81%) had a performance score of 2 or better (table 1). Most patients had undergone prior surgery (67%) and prior chemotherapy (74%). Over one-half of the patients (81%) had lung or pleural metastases, and 31% of patients had intra-abdominal sites of metastases as determined by ultrasound, computed tomography, and physical examination. All patients who were correctly entered in this study and received at least one dose of AMSA were considered evaluable.

Drug Administration

AMSA was initially administered at a dose of 60 or 120 mg/m^2, depending on the extent of prior chemotherapy and radiotherapy. Treatments were given every 3 weeks, and doses were increased by 20 mg/m^2 with each treatment until toxicity was encountered or a level of 160 mg/m^2 was achieved.

RESULTS

Response

Of the 42 patients evaluable for response, there were one with improvement for 2 months and seven with stable disease for at least 6 weeks. The 34 other patients had progressive disease. The median survival from start of treatment was 3+ months (range, ½ to 23+), but 23 of the 42 patients were still alive at the time of this determination. Ten patients received only one dose of drug, 11 patients received two doses, and 21 patients received three or more doses. Of the 42 patients, 26 received at least one AMSA dose of 120 mg/m^2, and five patients had doses escalated to the 160-mg/m^2 level.

Drug Toxicity

Clearly, the dose-limiting toxic effect was myelosuppression (table 1). Leukopenia was life-threatening in seven patients (17%), and three died because of infectious complications. Prior treatment was probably more important than actual dose in causing this toxic effect, since

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TABLE 1.—AMSA in hypernephroma: pretreatment patient characteristics and toxic effects

	No. of patients (%)
Evaluable	42
Male:female	24:18
Median age in yrs (range): 59 (33–80)	
Performance status	
Normal or minor impairment	24 (57)
Bedridden < 50% of the time	10 (24)
Bedridden > 50% but < 100% of the time	7 (17)
Bedridden 100% of the time	1 (2)
Extent of metastatic disease	
Lung or pleura	34 (81)
Lymph nodes	12 (29)
Bone	11 (26)
Liver	7 (17)
Skin	6 (14)
Brain	4 (10)
Other intra-abdominal sites	6 (14)
Other	3 (7)
Prior therapy	
None	3 (7)
Surgery	28 (67)
Chemotherapy	31 (74)
Single-drug	16 (38)
Combination of drugs	18 (43)
Hormonal therapy	21 (50)
Radiotherapy	17 (40)
Immunotherapy	4 (10)
Toxic effect	
Wbc count nadir (cells/μl)	
1000–2000	8 (19)
< 1000	7 (17)
Platelet count nadir (cells/μl)	
20,000–50,000	4 (10)
< 20,000	2 (5)
Degree of nausea and vomiting	
None	31 (74)
Mild	5 (12)
Moderate	5 (12)
Severe	1 (2)

these seven patients developed the severe leukopenia at all dose levels: 60 mg/m^2 (two patients), 120 (3), 140 (one), and 160 (one). Two patients (5%) had life-threatening thrombocytopenia, and one died of hemorrhage. Other toxic effects were generally mild, with 26% of patients having nausea or vomiting and 4% of patients having stomatitis. No hepatic, renal, or cardiac toxicity due to the drug was encountered.

DISCUSSION

This phase II study shows that AMSA in myelosuppressive doses is not active in renal cell carcinoma. This result is in agreement with another study (8) published after the start of ours. For the most part, the drug was well-tolerated in the dose range used; however, caution is warranted in patients with heavy prior treatment because of the possibility of severe myelosuppression in these patients.

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Maytansine in Refractory Childhood Acute Lymphocytic Leukemia: A Pediatric Oncology Group Study[1,2]

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We report on a trial of maytansine in children with acute lymphocytic leukemia (ALL) conducted by the Pediatric Division of the Southwest Oncology Group, now the Pediatric Oncology Group (1,2).

METHODS

Patients were eligible who were ⩽ 20 years of age and had leukemia in relapse (ie, ⩾ 25% blasts). The patients had not received prior therapy with maytansine and were resistant to conventional therapy.

Maytansine (0.6 mg/m^2) was administered by rapid iv infusion daily for 3 consecutive days. A complete response was defined as < 5% blasts in the bone marrow and a partial response was defined as > 5% and < 25% blasts.

RESULTS

Patient Population

A total of 17 patients were entered in the study. Two patients had acute nonlymphocytic leukemia, exhibited no response to therapy, and were not included in the tumor response analysis. Fifteen patients with ALL completed a full course of therapy. The mean number of previous relapses was 3.8.

Tumor Response

No complete or partial responses were observed.

Toxicity

Seventeen patients were evaluable for toxicity. Three patients did not report toxicity. One patient had an otherwise unexplained fever that was attributed to maytansine. Two patients had jaw pain which responded to analgesics. Four instances of moderate nausea and vomiting were recorded and four episodes of diarrhea were noted.

DISCUSSION

The iv administration of maytansine at the dose level and schedule utilized (3) did not show antitumor activity in advanced childhood ALL resistant to conventional therapy. Our finding of minimal toxicity in this study suggests that age-oriented phase I studies could result in different patterns of dose-related toxicity and/or different suggested starting doses for pediatric phase II studies.

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Phase II Clinical Trial of L-Alanosine in Advanced Upper Aerodigestive Cancer[1,2]

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L-Alanosine, an antibiotic produced by *Streptomyces alanosinicus*, has antiviral and antitumor activity in animal tumor models (1).This agent appears to exert cytotoxicity through its intermediary metabolites by inhibition of adenylosuccinate synthetase (2). From phase I investigation, an iv regimen of 250 mg/m^2 on Days 1-3 every 3 weeks produced stomatitis as dose-limiting toxicity (3). We report here a phase II study in 26 patients with biopsy-proven upper aerodigestive cancer (table 1).

L-Alanosine was administered iv over 10 minutes on each of 3 consecutive days every 3 weeks at a dose of 250 mg/m^2 diluted in normal saline to a concentration of 10 mg/ml.

There were no objective regressions of either measurable or evaluable disease using conventional response criteria (4). Fifteen patients (58%) demonstrated disease progression after only one course of treatment; 11 patients remained stable. Median time to disease progression was 1 month for all patients, while the estimated median survival was 3.7 months.

Side effects were primarily gastrointestinal. Among 26 patients, stomatitis occurred in ten (mild, four; moderate, five; and severe, one); nausea in nine (mild, six; moderate, two; and severe, one); and vomiting in four (mild, three; and severe, one). Among 18 patients with interim hematologic data, seven (39%) had a wbc count nadir < 4100/mm^3 and three had a platelet count nadir < 130,000/mm^3. We did not detect clinically significant hepatic or renal toxicity.

We could not demonstrate any therapeutic value for these patients with advanced head and neck cancer from the dose and schedule of L-alanosine which was used. Sixteen patients had squamous cell histologies and it is unlikely (< 5%) that the true regression probability is at least 20% in such patients. We are less secure in defining the activity of L-alanosine in patients with non-squamous cell histologies, but we noted no evidence of activity. The absence of regressions among ten patients is inconsistent with a 25% response rate ($P = 0.056$).

TABLE 1.—Patient and tumor characteristics

Characteristic	No. of patients
Male	20
Female	6
Eastern Cooperative Oncology Group performance status	
0,1	13
2,3	13
Prior chemotherapy	24
Prior radiation therapy	20
Carcinoma	
Squamous cell	16
Non-squamous cell	10

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Treatment of Side Effects in Patients Receiving Iv Metoclopramide

We read with interest the letter by Bui et al (1) reporting a significant frequency of severe extrapyramidal side effects (EPS) in patients receiving iv metoclopramide (MCP) to prevent chemotherapy-induced emesis. Iv MCP was stopped in eight of 25 (32%) of their patients because of problems with EPS. The dose of iv MCP employed in their series was 1.0 mg/kg of body weight for two doses. In contrast, other studies have used iv MCP at doses of 2.0 mg/kg for five doses (2) or 1.0 mg/kg for six doses (3). The frequency of severe EPS reported in these studies was 5% and 3%, respectively. In the latter of these studies (3), we reported our experience using iv MCP in 38 patients receiving cisplatin-containing chemotherapy, and observed only one instance of torticollis, which responded promptly to a single 50-mg dose of iv diphenhydramine. Akathisia or restlessness, a mild form of EPS, was seen by us much more frequently (seven patients, [18%]), and in only three instances did patients require diphenhydramine treatment. In most cases, akathisia was found to be related to the rate of MCP infusion, and simply slowing the infusion rate frequently resulted in resolution of this EPS. In > 3 years of using iv MCP for both inpatients (receiving cisplatin with iv MCP doses ranging from 1.0 mg/kg for six doses to 3.0 mg/kg for three doses) and outpatients (receiving non-cisplatin-containing regimens with iv MCP doses ranging from 0.3 mg/kg for three doses to 1.0 mg/kg for three doses), we have never had to stop iv MCP treatment in any patient because of EPS. In a recent series, we used iv MCP to prevent nausea and vomiting in 50 consecutive inpatients and outpatients receiving emetogenic non-cisplatin chemotherapy and achieved a 78% incidence of total antiemetic protection (no emesis) (39 of 50 patients). Iv MCP was given at a dose of 0.75 mg/kg for a median of three doses (range, two to six), with the first dose given ½ hour before the start of chemotherapy. Statistically, we could show no advantage of using more than three doses of iv MCP to prevent emesis, but did show more side effects (62% overall) with the higher dose (4). However, only one instance of severe EPS was noted (2%), which responded to one dose of iv diphenhydramine. Outpatients tolerated this dose of iv MCP extremely well, and no patient was unable to drive home from the office. In a more recent pilot study, we have employed a combination of iv MCP (0.5 mg/kg) and iv dexamethasone (10 mg), both given ½ hour before chemotherapy, followed by oral doses of each drug at fixed intervals after chemotherapy. This approach has resulted in 100% antiemetic protection in eight outpatients receiving potentially emetogenic noncisplatin chemotherapy. All patients are fully functional after therapy and no severe EPS has been noted. EPS, such as akathisia, have been observed most frequently in male patients < 50 years of age (approximately 20% incidence), but have also been noted occasionally in patients as old as 65 years of age. These reactions respond well to single or multiple doses of diphenhydramine. It has been our practice to routinely premedicate patients ≤ 40 years of age with diphenhydramine if they are to receive iv MCP doses ≥ 2.0 mg/kg. In outpatients receiving doses of 0.5–0.75 mg/kg, premedication has been required in only three of 43 patients (7%) who experienced EPS (all akathisia) with their initial exposure to MCP. One patient responded to a dose reduction of iv MCP to 0.3 mg/kg without the need for prophylactic diphenhydramine and still achieved total antiemetic protection. When the use of diphenhydramine has been required, the only side effect noted has been accentuation of drowsiness inherent to the use of MCP itself. With the single 50-mg dose that is generally required with outpatient therapy, drowsiness has never been incapacitating.

Bui et al state that comparable response rates with MCP can be achieved with lower doses than have been recently reported, and they compare their results with those of Gralla et al (2). We find it difficult to directly compare these two study groups, because patients treated by Gralla et al received high-dose cisplatin therapy (120 mg/m^2), whereas patients treated by Bui et al received cisplatin-containing regimens (75–120 mg/m^2) and non-cisplatin-containing regimens. Gralla et al demonstrated complete protection from nausea and vomiting with iv MCP (2 mg/kg for five doses) in eight of 21 (38%) patients, while Bui et al showed an overall complete protection rate of 27.5% when both cisplatin-treated and non-cisplatin-treated patients are combined. In our study using iv MCP to prevent emesis in noncisplatin regimens (4), we showed a 67% incidence of total antiemetic protection in a 21-patient subset receiving what we consider "highly emetogenic" agents which include dacarbazine, dactinomycin, mechlorethamine, or cyclophosphamide (≥ 1200 mg/m^2). The dose of iv MCP used with most of these patients was 0.75 mg/kg for three doses. We would therefore disagree with Bui et al that their dose scheme for iv MCP is as effective as the schedules currently in print, but we would agree that lower doses of iv MCP may be ef-

fective, especially with non-cisplatin-containing chemotherapy regimens.

We concur with Bui et al that the younger age population probably represents a different set of circumstances with respect to the type and occurrence of MCP side effects. Current phase I studies with iv MCP in younger patients, combined with the use of prophylactic diphenhydramine, have demonstrated the effectiveness of this approach in preventing the emergence of EPS and allow iv MCP to be used to prevent chemotherapy-induced emesis in these patients with successive courses (5).

After > 3 years of experience with MCP, it is our impression that this drug represents the single most effective and versatile antiemetic available to date to prevent chemotherapy-induced emesis. Side effects have been of low order in terms of frequency and severity and have been preventable or treatable with diphenhydramine.

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Stevens-Johnson Syndrome Associated With Etoposide Therapy

Etoposide, which is now widely used in the treatment of small cell lung carcinoma, testicular tumors, and malignant lymphoma, frequently causes bone marrow suppression, alopecia, and gastrointestinal symptoms. However, cutaneous hypersensitivity reactions have not been reported with its use (1-3). Here we describe a patient with Stevens-Johnson syndrome, which was probably caused by etoposide.

CASE REPORT

A 53-year-old white man with limited-stage small cell lung carcinoma began treatment with iv vincristine (2 mg), methotrexate (120 mg), and 5-day administration of etoposide (100 mg/day), without untoward reactions. Vincristine was administered weekly. On Day 22, he received cyclophosphamide (2 g) and doxorubicin (120 mg). The only other drugs he received during this period were acetaminophen plus codeine for painful neurotoxic effects and Compazine before each cycle of chemotherapy. On Day 43, he again received methotrexate (120 mg) and etoposide (100 mg/day for 5 days). Beginning on the third day of etoposide administration, the patient developed a sore throat and mild fever (100°F), which persisted through the fifth day of treatment. Four days following completion of etoposide administration, the patient returned with a 3-day history of rash on his neck, more severe sore throat, painful mouth sores, fever, weakness, and cough productive of yellow sputum but no arthralgia. He appeared acutely ill, with a temperature of 99.6°F and a diffuse maculopapular eruption over his neck, trunk, axillae, groins, palms, and soles, with frequent vesicles and ulcers in the center of the macules. The patient had no lymphadenopathy. His mouth and pharynx were diffusely involved with friable, bleeding ulcers with white plaques. His lungs were clear, and abdominal examination was unremarkable. Wbc count was 3300/μl, hemoglobin was 12.8 g/dl, platelet count was 50,000/μl, and prothrombin time and partial thromboplastin time were normal. BUN was 74 mg/dl and serum creatinine was 3.7 mg/dl (pretreatment values, 11 and 1.2 mg/dl, respectively). Urinalysis showed 0-2 rbcs and 2-4 wbcs, but no proteinuria. A chest x-ray showed the right lower lung mass to be improved from previous studies. There were no new infiltrates. Blood and urine cultures were sterile, but throat and sputum cultures grew *Candida albicans*. A monospot test was negative. Two punch skin biopsies showed histologic features of toxic epidermal necrolysis.

The patient was treated with nafcillin, tobramycin, and methyl prednisolone. Mouth pain necessitated parenteral hyperalimentation. The hospital course was further complicated by the worsening of pancytopenia, nausea, vomiting, diarrhea, and abnormalities of previously normal liver chemistries (highest values: alkaline phosphatase, 262 IU/ml; SGOT, 90 IU/ml; serum bilirubin, 4.0

mg/100 ml; and LDH, 386 IU/ml). Beginning on the fourth day in the hospital, the skin rash began to fade and the fever began to subside, but the mouth sores were slower to heal. Azotemia was corrected with hydration. Platelet count was maintained at > 10,000/μl with platelet transfusions. At the time of discharge on the 16th day in the hospital, the skin lesions had healed and mucosal ulcers had improved but were still present, wbc count was 15,000/μl, and platelet count was 167,000/μl. The mucosal ulcers gradually healed and the liver chemistries normalized over the next 4–6 weeks. The patient has since been treated with cyclophosphamide, doxorubicin, and vincristine, as well as acetaminophen plus codeine and Compazine, without ill effects. Further etoposide was not given.

DISCUSSION

The close temporal relation of the skin reaction to etoposide administration, combined with the rarity of such skin reactions to low- and moderate-dose methotrexate and the lack of bacteriologic evidence for a systemic infection, points to etoposide as the most likely cause of the skin reaction. However, the association must be considered presumptive in the absence of any direct evidence. We report this case merely to draw attention to this possibility.

Erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis constitute a continuum of increasingly severe skin and mucous membrane reactions. It is therefore not surprising that the clinical and histologic features of these entities often merge, as in our patient. Pathogenesis remains unclear, but an antibody response to the inciting agent and subsequent formation and deposition of immune complexes (hypersensitivity type III) in the cutaneous vessels seems likely in some patients. The development of the skin reaction soon after the second exposure to etoposide would be compatible with such a mechanism in our patient. The abnormalities of liver chemistries most likely reflect fibrinoid necrosis in the liver, which is sometimes associated with these entities.

Teniposide is closely related to etoposide and frequently causes anaphylactic reactions with hypotension and bronchospasm (4). Recently, an instance of immune hemolytic anemia mediated by an IgG antibody to teniposide has been described (immune complex or type III hypersensitivity) (5), showing that the drug is capable of inducing antibody formation. The minor variation in chemical structure between the two drugs would seem unlikely to preclude similar potential for etoposide.

Hypersensitivity reactions to chemotherapeutic drugs are being increasingly recognized; the subject has been recently reviewed (4).

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High-Dose Tamoxifen in Metastatic Melanoma[1]

The study of hormonal therapy in metastatic melanoma was stimulated by two observations. First, hormonal receptors were found in tissue specimens of melanoma (1). Second, observation of the natural history of the tumor suggested that hormonal influences might have a role in the growth and development of melanoma; ie, during adolescence, pregnancy, and menopause, a change in growth patterns of melanoma is not infrequent (2).

Several studies of hormonal therapy show results ranging from no response to brief partial response with antiestrogen, as well as evidence of tumor regression with estrogen compounds (3,4). While some studies show apparent high affinity of melanoma tissue for estrogen-binding protein (1), estrogen receptor levels are generally low when detected.

We therefore initiated a clinical trial of high-dose tamoxifen in patients with disseminated malignant melanoma. Thirteen patients were included in the assessment; estrogen receptor was assayed in all but one of the patients.

[1]Supported in part by funds from ICI Americas, Inc.

The procedure is reported elsewhere. Briefly, receptor analysis, performed in triplicate, is based upon cytosol assay of tritiated estradiol, separating bound radioactivity from free radioactivity on polyacrylamide gel filtration columns. Specificity of binding was examined by the addition of diethylstilbestrol to replicate samples.

Patients received 100 mg/m^2/day of tamoxifen orally in two divided doses until disease progression developed. Prior to treatment and at monthly intervals, appropriate x-rays and radionucleotide scans were performed. Routine hematology and blood chemistry tests were carried out weekly.

TABLE 1.—Characteristics of 13 patients with melanoma treated with tamoxifen

Characteristic	No. of patients
Evaluable	13
Median age in yrs (range): 53 (23–64)	
Males/females	8/5
Prior chemotherapy	13
Performance status*	
0	2
1	4
2	4
3	3
Dominant metastatic site	
Subcutaneous nodules	5
Lymph nodes	2
Lungs	5
Liver	1
Results	
Tumor regression	1 (3 wks)
Stable disease	4 (5 wks, 2 mos, 3 mos, 5 mos)
Disease progression	8
Side effects	
Fluid retention	1
Nausea	2

* Eastern Cooperative Oncology Group criteria.

One patient had regression of subcutaneous nodules for 3 weeks, and four patients had disease stabilization from 5 weeks to 5 months (table 1). The one patient who experienced regression of subcutaneous nodules did show the presence of estrogen receptor in tumor tissue. All other patients demonstrated disease progression. Side effects were not significant and consisted of fluid retention in one patient and mild nausea in two patients.

The receptor assays showed three patients with estrogen receptor, three with progesterone, and four with negative receptor studies. There was no correlation between presence or absence of receptor and response to tamoxifen.

The response to tamoxifen in advanced malignant melanoma is generally not impressive. In our study, the administration of higher doses did not yield appreciable improvement in the response rate, nor did it yield an increase in side effects, which proved to be negligible. Certainly no dose-response data for the use of tamoxifen exist.

The studies of McCarty et al (5) suggest that the presence of the enzyme tyrosinase or other substances may mimic estrogen receptor binding, so that an apparent affinity for estradiol is demonstrated. Based on these data, the presence of specifically inhibitable receptor-like estrogen binding could possibly be an artifact in melanoma tissue.

The occasional regression of melanoma with hormonal treatment is intriguing, however, and remains unpredictable.

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Third Winter Symposium on Hematologic Malignancies

Snowbird, Utah, February 18–25, 1984

For information, contact: Dr. Stephen E. Jones, Section of Hematology and Oncology, University of Arizona Cancer Center, Tucson, AZ 85724.

Intra-Arterial and Intracavitary Chemotherapy 1984

San Diego, California, February 24–25, 1984

This continuing medical education conference will take place at the Holiday Inn at the Embarcardero (San Diego, CA). The conference is approved for 14 hours of AMA/CMA credit as well as AAFP and the fee is $250.

For more information, contact: Office of Continuing Medical Education, UCSD School of Medicine, M-017, La Jolla, CA 92093.

Eighth Annual Symposium of The Cancer Research Center

Chapel Hill, North Carolina, March 15–16, 1984

This symposium, The Impact of Biotechnology on The Immunobiology of Cancer, will include sessions on, Monoclonal Antibodies in Cancer: Fact, Fancy, and Future; Cloned Cell Lines as Immunological Probes; and Molecular Biology for Fun and Profit.

For further information, contact: Ms. Pam Upchurch, Cancer Research Center, Box 30, MacNider Bldg, Chapel Hill, NC 27514 (telephone: 919-966-3036).

Tenth International Cell Cycle Conference

Banff, Alberta, Canada, April 24–28, 1984

The International Cell Cycle Society is holding its tenth conference at the Banff Center. Symposium topics will include the biochemistry and physiology of the cell cycle, chromatin and nuclear structure, genetics of the cell cycle, cell differentiation, fine structure of the cell cycle, and proliferative dormancy and activation.

For more information, contact: Dr. Susan Friedman, The Oncology Research Group, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Fellowship in Medical Oncology, Hematology, or Combined Oncology-Hematology

July 1984 and July 1985

Applications are now being accepted for a fellowship program involving major teaching hospitals of Brown University's Program in Medicine, beginning July 1984 and July 1985. Clinical activities and opportunities for research are offered to qualified candidates with two years of approved medical residency and ABIM eligibility.

For further information, contact: F. J. Cummings, MD, Director, Division of Medical Oncology, or Manfred Steiner, MD, Director, Division of Hematology, Brown University, Box G, Providence, RI 02912.

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